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CHARLES ELMER OWENS
1877—1957

Howard P. Barss and Floyd D. Bailey

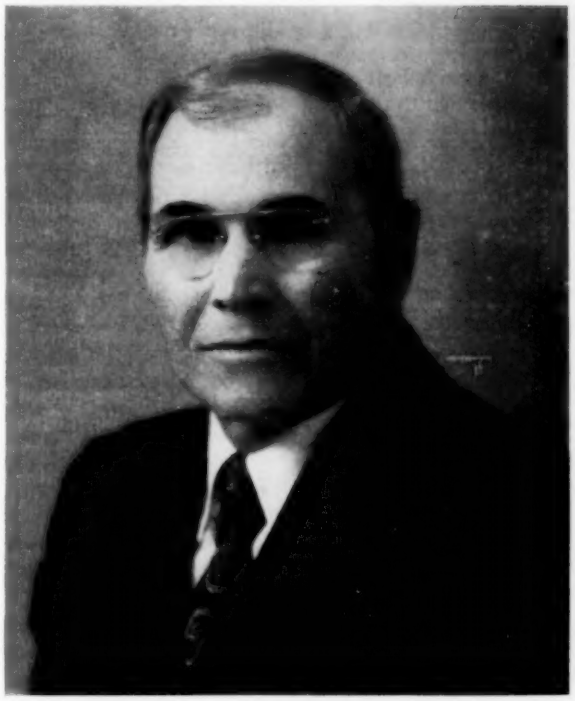
Charles Elmer Owens, 79, head of the Department of Botany and Plant Pathology at Oregon State College from 1934 to 1947, and author of "Principles of Plant Pathology," died in Corvallis, Oregon, May 1, 1957. During his 35 years at the College, Dr. Owens contributed much to the development of plant pathology on the Pacific Coast by his exceptionally effective teaching, his scholarly textbook, and his friendly helpfulness to students and associates. In 1930–31 he represented the Pacific Division on the Council of the American Phytopathological Society.

Born in Clay County, Indiana, November 26, 1877, to Elias D. and Anna Owens, Charles grew up and worked on the family farm. He finished 2 years of high school at nearby Harmony. At 23 he entered the State Normal School, where he financed himself by intervals of public-school teaching. He worked his way through Indiana University, specializing in botany and plant pathology under the inspiring teacher David M. Mottier, and serving 2 years as teaching fellow. He received his A.B. in 1910, and his A.M. in 1911. Graduate studies later led to his Ph.D. in 1934 at the University of Wisconsin.

Dr. Owens joined the staff of Oregon Agricultural College in 1912, as instructor in botany. He rose eventually to full professorship, and from 1934 to his retirement, in 1947, he served as head of the Department of Botany and Plant Pathology in the College and Experiment Station. During his administration the Department continued to expand its program of instruction and research. His sympathetic attitude led to further cooperation with other states and the U. S. Department of Agriculture on important plant disease problems. In World War I days he served part time in the Federal Plant Disease Survey. Following retirement he carried on, for an industrial firm, investigations of alleged smelter-fume damage to crops. For some months before his death he was engaged in part-time teaching, research, or extension work for the College, much to his enjoyment.

In 1913 he married Elizabeth Underwood, a fellow student in the Normal School and an able teacher who did much for public school education in Corvallis. She survived him by only a few months. There were no children.

Although Owens possessed the technical skill, precision, and persistence for good research, his opportunities for research were limited. Nevertheless he found time to help C. W. Hungerford in studies of stripe rust of cereals, and S. M. Zeller in work on European canker. His investigations of wood-rotting fungi included *Fomes pini*, the subject of his doctoral thesis, published in 1936 in the American Journal of Botany. A number of popular circulars were also issued under his authorship.



CHARLES ELMER OWENS
1877–1957

It was through his rare effectiveness in the classroom that Owens made his outstanding contribution to the advancement of his chosen field. His deep interest in his students as well as his subject, along with his clear and orderly presentation, marked by a quiet enthusiasm and gentle sense of humor, inspired many of his students to elect plant pathology as a career, in which they have made notable contributions to the benefit of agriculture and forestry. For use in his elementary and advanced courses Dr. Owens built up one of the best collections of plant-disease specimens on the Pacific Coast, including the major timber-destroying fungi of concern to the forestry of the region. His textbook, "Principles of Plant Pathology," published in 1928, included for the first time a number of plant diseases of special importance on the Pacific Coast, meeting a long-felt need.

Dr. Owens was an able and dedicated member of the underpaid and seldom-honored profession of science teachers, whose true importance to the nation and the world is now being tardily recognized. His former students and associates pay high and affectionate tribute to his memory.

PHYTOPATHOLOGY for May (48: 235-290) was issued May 30, 1958

THE ASSOCIATION OF OLPIDIUM WITH THE BIG-VEIN DISEASE OF LETTUCE[1]

R. G. Grogan, F. W. Zink, Wm. B. Hewitt, and K. A. Kimble

SUMMARY

Symptoms of big vein did not develop in lettuce grown in previously infested soil that had been treated with DD, chloropicrin, CBP 55, or CS_2, but did develop in plants grown in infested soil that had been treated with EDB. Symptoms did not develop when plants were exposed to big-vein-diseased roots that had been surface-sterilized with several fungicides; nor did symptoms develop in plants that were grown in big-vein-infested soil for 8 weeks, dug, dipped in $HgCl_2$ solution, and transplanted to sterilized soil. Symptoms developed in plants exposed to superficial scrapings from roots of big-vein-diseased plants, but did not develop in plants exposed to the interior portions of the same roots. Examination of roots from typically diseased plants from different locales in California, Arizona, Utah, and New Zealand proved that all were consistently infected with *Olpidium brassicae* (Wor.) Dang. Lettuce seedlings exposed to *Olpidium*-infected roots of lettuce or other hosts developed big-vein symptoms. When suspensions of *Olpidium* zoospores were filtered through filters that were capable of retaining the zoospores but able to pass a virus, the filtrates did not induce big-vein symptoms. These results indicate a causal relationship between *Olpidium* and the big-vein disease, which is not contradicted by any proof of virus etiology in the extensive literature on the disease.

Although the cause of the big-vein disease of lettuce, *Lactuca sativa* L., is commonly considered to be a soil-borne virus, a critical review of the literature has failed to reveal any real proof of this relationship. The disease was first reported in 1934, by Jagger and Chandler (6), who had observed it since 1929. Because big vein occurred with the brown blight disease (5), it had not been recognized as a distinct disease until lettuce varieties resistant to brown blight were developed. These authors reported big vein to be infectious and soil-borne, similar to the soil-borne wheat mosaic, but claimed no definite proof of virus etiology. They further noted the presence of *Olpidium* in the roots of brown-blight-diseased plants, a significant point considering the observations reported in the present paper. Rawlins and Tompkins (11), in 1936, failed to transmit big vein by mechanical inoculations using Carborundum and juice from the leaves of big-vein-infected plants. They stated that the disease had not been proven to be caused by a virus. Thompson and Doolittle (16), in 1942, presented evidence that symptoms of big vein develop at lower air temperatures (50–60°F during the day and 45–50°F at night), but not at higher temperatures (65–75°F during the day and 50–60°F at night). No statement was made relative to the nature of the causal agent.

Pryor (9), in 1944, reported that more plants developed big-vein symptoms when grown at a high soil-moisture level. He was unable to explain this observation because the nature of the causal agent had not been demonstrated. He was not certain whether increased soil moisture accentuated symptom expression by an effect on the host, the causal agent, or the disease complex. In 1944 Thompson, Doolittle, and Smith (17) suggested that big vein might be spread by the root aphid *Pemphigus lactucae*. However, the authors said the evidence concerning transmission by the root aphid was only circumstantial, and, if the root aphid was a vector, it was not known whether the virus was carried internally and spread by feeding or externally through contact with infested soil. This paper was the first to designate a virus as the causal agent of big vein. Doolittle and Thompson (3), in 1945, reported that mechanical inoculations with juice expressed from big-vein-infected leaves failed, but that infection was readily obtained when juice from roots was rubbed onto leaves or pricked into leaves or stems. This report undoubtedly started a trend of thinking that big vein is caused by a virus restricted to the roots of diseased plants. This account of transfer to lettuce foliage was nullified in 1948, by Allen (1), who reported that symptoms of big vein did not develop when leaves were rubbed with juice from roots of infected plants if precautions had been taken to prevent the inoculum from reaching the soil. Pryor (10), in 1946, reported on investigations of several factors concerning the nature of the causal agent of big vein. Attempts to transmit the causal agent by insects, by mechanical inoculations of juice from leaves of infected plants, or by seed gave inconsistent and mostly negative results. He also reported that the causal agent survived in air-dried soil for 8 years and would not pass through a medium Mandler filter.

Fry (4), in 1952, and Yarwood (19), in 1954, reported the association of tobacco necrosis virus with the roots of big-vein-infected plants, but neither author has reported big-vein symptoms following inoculation with this virus. Several other papers (2, 12, 13) on the big-vein disease have been published that do not discuss the nature of the causal agent. One must conclude that the nature of the causal agent has not been demonstrated and that no one has recorded proof of virus etiology.

This paper analyzes several characteristics of the causal agent and presents evidence that the disease is closely associated with root infection by the fungus *Olpidium brassicae* (Wor.) Dang.

[1] Accepted for publication December 30, 1957.

EXPERIMENTAL METHODS.—Lettuce plants of the variety Imperial 615 were used in all experiments. Plants grown in steam-sterilized vermiculite were transplanted to steam- or chloropicrin-sterilized soil or steam-sterilized quartz sand watered with nutrient solution. In all experiments 2 types of controls were included: 1) plants inoculated by growing them in soil containing root material from big-vein-diseased plants, to ascertain whether environmental conditions were suitable for symptom expression, and 2) uninoculated plants growing in sterilized soil to determine whether chance infections had occurred during the experiment. To prevent chance contaminations, care was taken to avoid splashing from one pot to another during watering, and insecticidal sprays were periodically applied to prevent insect infestation and movement from pot to pot.

EXPERIMENTAL RESULTS.—*Effect of soil fumigation.*—Big-vein-infested soil was fumigated with DD (dichloropropene dichloropropane), EDB (ethylene dibromide), chloropicrin, CBP 55 (chloro bromo propene), or carbon disulfide by injecting the chemical into the center of the soil mass, in a 2-gal. container, at the rate of 1 g of active ingredient per 11.5 lb. (gal.) of soil. The containers were then kept closed for 72 hours, after which the soil in each was divided among 10 3-inch pots and allowed to aerate for 14 days. Then 1 lettuce plant was transplanted to each pot. After 50 days no symptoms had developed on plants in soil treated with DD, chloropicrin, CBP 55, or carbon disulfide, but symptoms had developed in 6 of 10 plants in EDB-treated soil and 7 of 10 in untreated soil.

Surface-sterilized roots from affected plants as inoculum.—Roots from big-vein-diseased plants were washed in tap water to remove visible dirt and other extraneous material and dipped for 10 minutes in solutions of various fungicides. Dipped roots were rinsed thoroughly in tap water and chopped up with a sterile scalpel in individual sterile Petri plates. Three g of these chopped roots were placed around the crown of each healthy seedling. A representative sample of the data obtained (Table 1) shows that development of big-vein symptoms was prevented by surface sterilization with Clorox (5¼% NaOCl), $HgCl_2$, and Semesan, but not by treatment with Vancide, Nabam, or Captan.

Treating whole plant with mercuric bichloride.—Symptomless lettuce plants that had grown in big-vein-infested soil for 8 weeks were dug and all visible soil washed from the roots. The whole plant was then immersed in 1:2000 $HgCl_2$ solution for 10 minutes, rinsed in water, and transplanted to steam-sterilized soil. Eight of 10 plants survived the treatment and none had developed big-vein symptoms 79 days after transplanting. Eight of 10 plants treated the same but not dipped in $HgCl_2$ also survived, and 5 of them had developed big-vein symptoms 79 days after transplanting. Nine of 10 plants transplanted to sterilized soil without root washing or dipping survived, and 7 of these plants had developed big-vein symptoms 79 days after transplanting. This experiment shows that surface-sterilization of the plant prevented big-vein symptom development even though the sterilant did not penetrate deeply enough to kill the plants. This is further evidence that the cause of big vein is superficially borne.

TABLE 1.—*Number of plants that developed big-vein symptoms 65 days after inoculation by exposure to roots from big-vein-diseased plants that had been surface-treated with various chemicals*

Treatment of inoculum	Number plants	
	Inoculated	Diseased
Uninoculated control	10	0
Unwashed roots from BV plants	10	10
Washed roots from BV plants	10	10
BV roots treated with Clorox (5¼% NaOCl) (1:10 for 10 minutes)	10	0
BV roots treated with $HgCl_2$ (1:1000 for 10 minutes)	10	0
BV roots treated with Semesan (1:100 for 10 minutes)	10	0
BV roots treated with Vancide (1:100 for 10 minutes)	10	9
BV roots treated with Nabam (1:100 for 10 minutes)	10	6
BV roots treated with Captan (1:100 for 10 minutes)	10	4

Filtering the inoculum.—A number of tests were made to determine whether the causal agent of big vein would pass through filters of various pore sizes. In the earlier experiments, inoculum was prepared by grinding roots from big-vein-diseased plants and extracting the juice in a hydraulic press. Symptoms were produced consistently with unfiltered juice poured around the roots of healthy plants, but the juice was not infectious after passage through No. 5 or 7 Mandler filters. In later tests, inoculum was prepared by mixing big-vein-infested quartz sand with an equal volume of water and filtering through a double layer of cheesecloth. In 2 experiments, symptoms developed in practically all plants inoculated with 40 ml of this inoculum poured around their roots. The inoculum was infectious also after passage through a Pyrex fritted glass filter with 40-μ pore size, but was not infectious after passage through Pyrex fritted glass filters with pore sizes of 14.0, 5.0, or 1.2 μ.

Location of causal agent.—The prevention of symptom expression by chemical surface treatment of infested roots indicated that the causal agent was superficially in the roots, or possibly completely outside them. Ten roots from big-vein-diseased plants were washed thoroughly in tap water and the root surface scraped off with a scalpel, leaving the inner portions completely white. The inner portions were then washed thoroughly in a spray of water to remove any clinging external root fragments. The outer and inner portions were then placed separately around the crowns of healthy plants in sterilized soil. Three of 10 plants exposed to surface scrapings developed big-vein symptoms in 70 days; none of 10 plants exposed

to the inner portion of these same roots developed symptoms in the same period.

Cultures from, and microscopic examination of, roots from big-vein plants.—The causal agent's failure to pass through filters with relatively large pore sizes indicated that it might be large enough to be seen with an ordinary light microscope. Consequently, washed roots from big-vein-diseased plants were examined for organisms on or near the surface. The fine roots from all plants showing symptoms of big vein were heavily infected with *Olpidium brassicae* (Wor.) Dang. (18).[2] The fungus was not present in the roots of corresponding control plants. A variety of fungi and bacteria isolated in pure culture from roots of big-vein-diseased plants failed to produce the disease in lettuce seedlings in sterilized soil in the greenhouse. Since *Olpidium* is not culturable in the usual mycological media, it was not detected by routine isolation methods.

Following these observations, samples of roots from big-vein plants were secured from widely separated locations: Salinas, Milpitas, Sacramento, Oxnard, Santa Maria, and Delano, in California; Yuma, Arizona; Salt Lake City and Ogden, Utah; and New Zealand.[3] Without exception, the fine rootlets were heavily infected with *Olpidium*. Numerous other samples of roots from big-vein-diseased plants have since been examined, and all were infected with *Olpidium*. Roots from symptomless plants from fields containing high percentages of big-vein-infected plants were usually infected. In contrast, the fungus was not found in roots from plants obtained from fields where no big vein was evident. Thus the presence of *Olpidium* on roots of brown-blight- or big-vein-diseased lettuce, as previously noted by Jagger (5), was confirmed.

Those observation agree closely with those of Mr. P. R. Fry, of New Zealand, as reported in a letter to the senior author, June 6, 1957: "Olpidium is invariably present in large numbers in the fine roots of plants showing big-vein and to a lesser extent in non-big-vein plants from infected soil or plants inoculated with big-vein root sap but not developing symptoms. This holds for experimental plants and those collected in big-vein gardens throughout New Zealand. No Olpidium was present in roots of plants collected in the only lettuce growing district where I have not found big vein."

Further evidence of the association of *Olpidium* with big vein was presented by Jagger (5), who reported that *Asterocystis radicis* de Wildeman, which is synonymous with *Olpidium brassicae* (14), was found repeatedly and abundantly in the roots of brown-blight-diseased plants, but only rarely and scantily in healthy plants. The cause of brown blight has not been determined. It has occurred so consistently in association with big vein that at first the two were thought to be phases of the same disease (16). Jagger finally concluded that big vein was a distinct disease, for it occurred in selections that were brown-blight resistant. Since this does not actually prove that the two are different diseases, it indicates the desirability of re-examining the etiology of brown-blight.

Movement of causal agent through water.—To determine whether the cause of big vein can move through water, the following experiment was performed: A thoroughly washed tap root, including laterals, from a big-vein-diseased plant was placed in one end of a 4 × 10-in. glass refrigerator dish containing sterile distilled water ½-in. deep. At the opposite end of the dish, 20 lettuce seedlings were placed with their roots in the water about 5 in. away from the extremities of the big-vein-diseased root. Care was taken to avoid agitating the water. After 4 hours in the water, the seedlings were removed and transplanted to sterilized quartz sand. Six 20-plant groups were thus exposed to the single diseased root. Three additional 20-plant groups received 24-hour exposures on succeeding days, making a total of 9 groups, of 20 plants each, exposed to the big-vein-diseased root in the same dish during a 96-hour period. Data pertaining to big-vein symptoms and infection by *Olpidium* 120 days after inoculation, are presented in Table 2. The causal agent of big vein proved capable of mov-

TABLE 2.—*Number of plants showing big-vein symptoms 120 days after inoculation by exposure to roots from big-vein-infected plants across a barrier of water*

Time elapsed since big-vein-infected roots first placed in water (hours)	Exposure time of seedlings (hours)	No. of plants of 20 with big-vein symptoms	Root infection by Olpidium [a]
Uninoc. ck., planted from seedling pot at start of experiment	0	0	—
0	4	1	+
4	4	3	+
8	4	5	+
12	4	0	—[b]
16	4	5	+
20	4	2	+
24	24	8	+
48	24	1	+
72	24	1	+
Uninoc. ck., planted from seedling pot at end of experiment	0	0	—

[a] A composite sample of roots was mounted on a microscope slide and examined for sporangia and resting spores of Olpidium.

[b] Transplanting of these seedlings was inadvertently delayed. The roots were therefore drier than usual when transplanted.

[2] The writers are indebted to Dr. J. T. Barrett for confirming the identification of this organism.

[3] The writers express their appreciation to Dr. D. H. Hall, who collected the root samples from Ogden and Salt Lake City, Utah, and Santa Maria, California, and to Mr. P. R. Fry, who sent the root sample from New Zealand and allowed the writers to quote from his letter of June 6, 1957.

ing through the water and there was good positive correlation between symptom expression and root infection by *Olpidium*.

Correlation of big-vein symptoms with Olpidium infection.—Attempts by the authors to infect lettuce roots with *Olpidium* in the complete absence of other organisms were not successful. However, zoospore suspensions of *Olpidium* were rendered practically free of other organisms by the following method: *Olpidium*-infected lettuce roots, washed thoroughly with a strong spray of tap water for 5–10 minutes to remove soil and other extraneous material, were placed in sterile distilled water in Petri dishes for 10–15 minutes to allow the zoospores of *Olpidium* to be extruded. The infected root material was removed and discarded, leaving large numbers of zoospores in the water. Aliquots of the zoospore suspension, examined with a 4-mm objective, were found to contain only uniflagellate zoospores of a size, shape, and motility characteristic of *Olpidium brassicae* (14). Ten healthy lettuce plants growing in steam-sterilized soil were inoculated by pouring 25 ml of zoospore suspension around the crown of each plant. Typical symptoms of big vein developed in 7 of the plants by 60 days, and the roots of all plants were infected with *Olpidium*.

To further test the degree of correlation between big-vein symptom development and infection of roots by *Olpidium*, plants growing in steam-sterilized soil were inoculated either with zoospore suspensions or with root-derived inocula treated by several physical or chemical means. The purpose was to eliminate *Olpidium* in some treatments, maintain the inoculum potential in others, and lower it in others. Table 3 lists the various inocula treatments and data on big-vein symptom expression and *Olpidium* root infection. A causal relationship is strongly indicated between *Olpidium* infection of roots and big-vein symptom expression. Without exception, plants with symptoms of big vein had *Olpidium* infection in the roots. It is true that some plants with *Olpidium*-infected roots had not developed symptoms of big vein by the end of the experiment. For example, plants inoculated with zoospore suspensions that had been heated to 55°C for 10 minutes were symptomless although their roots were infected with *Olpidium*. The reasons for this discrepancy are not known, but the results obtained when inoculum was diluted with water or treated with sublethal doses of fungicides indicate that treatments that

TABLE 3.—*The effect of various treatments of inoculum upon big-vein symptom expression and Olpidium root infection*

Inoculum treatment	No. plants inoculated	Number of plants with big-vein symptoms after 47 days	54 days	60 days	67 days	Olpidium infection of roots after 67 days
Filtration [a]						
40 μ	22	10	11	16	20	22
14 μ	5	0	0	0	0	0
5 μ	5	0	0	0	0	0
1.2 μ	5	0	0	0	0	0
Dilution [a]						
10^{-1}	5	0	1	1	4	5
10^{-2}	5	0	0	2	3	4
10^{-3}	5	0	0	0	0	1
10^{-4}	5	0	0	0	0	0
10^{-5}	5	0	0	0	0	0
Heat treatment [a]						
40°C	5	2	3	3	4	5
45°C	5	3	4	4	5	5
50°C	5	3	4	5	5	5
55°C	5	0	0	0	0	4
60°C	5	0	0	0	0	0
70°C	5	0	0	0	0	0
Fungicide treatment [b]						
Panodrench 0.1 ppm	5	1	2	3	4	5
Panodrench 0.05 ppm	5	2	4	5	5	5
$HgCl_2$ 0.5 ppm	5	0	3	4	4	5
BV-diseased roots [c] dipped in						
1-1000 $HgCl_2$ for 2 min.	5	0	0	0	3	5
4 min.	5	0	0	0	0	3
8 min.	5	0	0	0	0	2
10 min.	5	0	0	0	0	1
Untreated BV diseased roots	5	2	2	5	5	5
Uninoculated check	12	0	0	0	0	0

[a] Inoculum was 25 ml of zoospore suspension.
[b] Panodrench or $HgCl_2$ was added to zoospore suspension in amounts sufficient to raise concentration of the active ingredients [cyano (methylmercuri) guanidine or Hg, respectively] to level indicated.
[c] 1.5 g of chopped roots were used as inoculum.

reduced the inoculum potential of *Olpidium* increased the time required for symptom expression. For example, plants inoculated with zoospore suspensions that had been diluted with water 10^{-1} developed symptoms earlier and in more of the plants than in the 10^{-3} dilution, wherein no symptoms developed but the root of 1 of the plants was infected with *Olpidium*. Treatments of the zoospore suspension with Panodrench 4 in a solution containing 0.05 ppm of the active ingredient [cyano (methylmercuri) guanidine] resulted in earlier symptom expression in more of the plants than did a similar treatment with the same material but at the higher concentration of 0.1 ppm. The $HgCl_2$-dip treatment of root inoculum affected symptom development similarly. Plants exposed to untreated roots from diseased plants were all infected with *Olpidium* and developed big-vein symptoms earlier and in more of the plants than did those that were exposed to similar roots that had been dipped for 2 minutes in 1:1000 $HgCl_2$. Although the comparable 4-, 8-, and 10-minute $HgCl_2$ treatments failed to eliminate *Olpidium* completely, there was a progressive reduction in the number of plants that became infected, and none of the plants in these treatments developed symptoms.

Zoospore suspensions filtered through Pyrex fritted glass filters with pore sizes of 14 μ or smaller failed to produce any *Olpidium* root infection or big-vein symptoms. In contrast, inoculation with 25-ml aliquots of zoospore suspensions that had been filtered through a 40-μ Pyrex fritted glass filter consistently produced *Olpidium* root infection and big-vein symptoms. One might expect the zoospores of *Olpidium* to pass through the 14-μ filter, because the average diameter of their heads is 4–5 μ. In fact, a few zoospores do pass through this filter, but, apparently, not enough to produce consistent infection, because, in 2 separate trials, no infection developed in roots inoculated with zoospore suspensions that had been passed through a 14-μ filter. The perfect correlation obtained in the filtration studies is considered to be as strong evidence of a causal relationship between *Olpidium* root infection and big-vein symptom expression as can be presented until methods are developed for obtaining pure cultures of the organism.

Inoculation with Olpidium-infected roots from other hosts.—*Olpidium brassicae* has been reported to parasitize the roots of a number of different host plants. For example, Sampson (14) listed the following genera as susceptible hosts: *Brassica*, *Capsella*, *Thlaspi*, *Linum*, *Plantago*, *Veronica*, *Limnosella*, *Solanum*, *Avena*, *Triticum*, *Poa*, *Agrostis*, *Festuca*, and *Lolium*. In a limited survey of hosts from the lettuce-growing area near Salinas, California, the organism was found parasitizing the roots of *Apium graveolens* L. (celery), *Beta vulgaris* L. (sugar beet), *Raphanus sativus* L. (Radish), *Allium cepa* L. (onion), *Brassica oleracea* L. var. *italica* Plenck (broccoli), *Chenopidium murale* L., *Brassica campestris* L., *Senecio vulgaris* L., *Polygonum aviculare* L., *Poa annua* L., and *Lactuca serriola* L. None of these hosts had apparent symptoms at the time of collection. Roots from these hosts were washed thoroughly in tap water and placed around the crown of lettuce plants in steam-sterilized soil in the greenhouse. *Olpidium* infected the lettuce roots in all instances. Big-vein symptoms developed in lettuce that had been exposed to *Olpidium*-infected roots from celery, radish, onion, broccoli, or *L. serriola*, but not in lettuce exposed to *Olpidium*-infected roots from the other hosts. The apparent differences in symptom development may not be reliable, for these experiments were conducted while temperatures were too high for optimum symptom expression. Even so, it does seem significant that big-vein symptoms developed when *Olpidium*-infected roots of hosts other than lettuce were used as inocula.

Discussion.—The consistent association of *Olpidium* with the roots of big-vein-diseased lettuce plants, both in experimental tests and in widely separated field plantings, along with the development of big-vein symptoms after inoculations with zoospore suspensions of *Olpidium* from lettuce or with *Olpidium*-infected roots from other hosts, is strong evidence of a causal relationship.

The mode by which the abnormality is produced is not known. There is a possibility that *Olpidium* acts as a vector of a virus, but data obtained in this and previous studies fail to substantiate the theory.

Although big-vein of lettuce resembles in some respects the soil-borne mosaics of wheat and oats (8), it differs distinctly in that the leaves of diseased plants do not contain an infectious entity that is transmissible by mechanical methods.

There seems to be no valid reason for considering the virus theory more favorably than other possibilities in the absence of any good evidence that a virus is involved in the production of the symptoms of big vein.

The writers favor the theory that the symptoms of the big-vein disease are caused by some substance produced by *Olpidium* that induces symptoms after being translocated to the leaves. This is somewhat analogous to the mode by which *Periconium* produces symptoms in milo (7) and by which bacterial constituents of the soil microflora produce the symptoms of frenching in tobacco (15).

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MUTATION TO WIDER VIRULENCE IN MELAMPSORA LINI[1]

H. H. Flor

SUMMARY

F_1 urediospores of race 22 × race 1 of *Melampsora lini*, subjected to X ray, at LD_{50} and LD_{90}, were screened for mutations on 24 flax varieties. The genotype governing pathogenicity of the F_1 culture was postulated from selfing studies of the parent races and from the pathogenicity of F_1 hybrid cultures. It was homozygous for virulence on 5 varieties, homozygous for avirulence on 3, and heterozygous, with avirulence dominant, on 16. Based on the number of primary uredia that developed on susceptible varieties, each resistant variety screened about 200,000 viable spores for mutants. The varieties to which the F_1 culture was homozygous for avirulence were resistant to all mutants, as were 5 of the varieties to which the F_1 urediospores were heterozygous for pathogenicity. The number of mutations to virulence screened on other varieties was 2–49, totaling 154, of which 94 had sufficient vigor for pathogenicity tests. Of these, 92 differed from the F_1 culture in attacking a single additional variety or group of varieties. The other 2 each attacked 2 additional varieties or variety groups. The groups of varieties on which pathogenicity appeared to be conditioned by a unit mutation were the same as those on which pathogenicity was found to be linked in hybridization studies. Eight mutant races, 7 not encountered previously, were obtained. Two races were secured in a test for natural mutations. The naturally mutating genes were the same ones that mutated most frequently in response to X irradiation.

Interest in the production of disease-resistant mutants in crop plants has been stimulated by the increased availability of irradiation facilities at colleges, universities, and national laboratories of the Atomic Energy Commission. But in disease control, mutations to virulence in the pathogen are as important as mutations to resistance in the host. Plant breeders and pathologists are, unfortunately, familiar with the succumbing of newly developed disease-resistant varieties to races of the pathogen not previously encountered.

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A determination of the relative mutation rates of the genes for pathogenicity could have great value to the plant breeder. If he had a choice of resistance germ plasm for breeding resistance in the host, he could use those genes against which mutations to virulence in the pathogen occur in low frequencies.

Most rusts appear relatively stable in the uredial stage. A race of *Puccinia graminis* Pers. f. sp. *tritici* Erikss. & Henn. was maintained for more than 100 urediospore generations with no change in pathogenicity (9). The writer observed no mutants while maintaining a number of races of *Melampsora lini* (Pers.) Lév. for 25 years, with at least 2 uredial generations each year.

But rust mutations have been reported for both spore color and pathogenicity. Changes in spore color are readily seen, and a number have been observed

(11). Mutations for pathogenicity have been reported less frequently, possibly because of the difficulty of positively differentiating mutants from contaminants. Three different patterns of pathogenicity mutations have been reported: 1) a number of separate mutations in a urediospore culture of one race (13); 2) repeated occurrence of the same mutation in the urediospore culture of a race (8); and 3) gradual replacement of a known race by a previously unknown one (10). The genetics of pathogenicity of the rust fungi involved in these mutations were not established.

Flor (6) secured 4 different virulence mutations following ultraviolet irradiation of urediospores of the flax rust fungus. Schwinghamer (12) observed a spore-color variant as well as a high frequency of virulence mutations on 2 varieties of flax following exposure of urediospores of race 1 of *M. lini* to X rays, neutrons, and ultraviolet light.

This paper reports X-ray-induced and natural mutations to virulence in an F_1 urediospore culture of the flax rust fungus. The genotype for pathogenicity was postulated from selfing studies of the parent races and from the pathogenicity of F_1 hybrid cultures. A preliminary report on the pathogenicity of some of the mutants has been published (7).

MATERIAL AND METHODS.—*Genetics of pathogenicity of urediospore culture.*—Host-parasite interaction between flax and the flax rust fungus has been explained on a gene-for-gene basis (1,2,3,5). For each gene in the host that conditions its reaction to rust there is a corresponding, or complementary gene in the parasite that conditions its pathogenicity. Pustule type, the criterion both of reaction in the host and pathogenicity in the parasite, is controlled by the interaction of these genes. In flax, resistance to rust has been dominant to susceptibility; in the parasite, avirulence has been dominant to virulence, with one exception (2). Twenty-five genes that condition rust reaction in flax have been identified, and lines of flax that apparently possess each gene have been developed (4).

Pycnia of the flax rust fungus are haploid and of two mating types. A pycnium is incapable of further development unless fertilized by pycniospores from a pycnium of complementary mating type. Then it develops into an aecium bearing dikaryotic aeciospores. Aeciospore infection gives rise to the dikaryotic uredium, the repeating phase. As there has been no nuclear fusion or reduction division in the aecial or uredial stages, one nucleus in each urediospore is identical to that of the pycnium from which the aecium developed (maternal); the second corresponds to that of the pycnium supplying the pycniospores (paternal).

Races 1 and 22, with the widest contrast in pathogenicity of the races available for this study, were selfed and crossed by methods that have been described (1). All the differential varieties except Bison, Williston Brown, Burke, and Ward were resistant to race 1; only Bombay and Stewart were resistant to race 22. Kugine, a variety of undetermined rust genotype that has not been used as a rust differential, was resistant to both races.

Selfing of race 22 indicated that it was homozygous for pathogenicity, a verification of previous studies (1). Selfing of race 1 showed it to be heterozygous for pathogenicity on flax varieties Akmolinsk, Dakota, Koto, and Victory A. The genes conditioning pathogenicity on Akmolinsk and Koto were either alleles or so closely linked that no crossing over occurred.

Hybrid cultures were secured by transferring pycniospores from single pycnia of race 1 developed on Bison to single pycnia of race 22 developed on Ottawa 770B. Aecia developed normally, but the resulting aeciospores and urediospores did not reinfect Ottawa 770B, the variety on which they were produced. This assured that the culture was a hybrid and not a self of race 22. Each of the 8 F_1 cultures secured attacked either Akmolinsk or Koto. Dakota and Koto have been either highly susceptible or practically immune from all races, while Akmolinsk and Victory A may have intermediate reactions to some races to which they are resistant. Consequently, a culture of race 22 $\times$ race 1, to which Dakota and Koto were immune and Akmolinsk and Victory A susceptible, was selected for study. This culture was homozygous for virulence on Akmolinsk, Bison, Burke, Victory A, Ward, and Williston Brown, and for avirulence on Bombay, Stewart, and Kugine. It was heterozygous for pathogenicity (avirulent) on Abyssinian, B. Golden selection, Barnes, Birio, Bowman, Cass, Clay, Dakota, Kenya, Koto, Leona, Marshall, Ottawa 770B, Pale Blue Crimped, Polk, Towner, and Wilden. The nucleus derived from race 1 carried the dominant genes conditioning avirulence; the nucleus derived from race 22 carried the recessive genes. The mutation of a functionally dominant gene to a recessive when present in the heterozygous condition in the rust spore should enable a spore to attack the differential variety carrying the specific complementary gene responsible for resistance to it.

Screening mutants.—Two 3-cc lots of urediospores produced on the variety Bison were subjected within a week to X ray LD_{50} and LD_{90}^{+} (55,000 *r* and 88,000 *r*, respectively) by E. A. Schwinghamer, at Brookhaven National Laboratory. About 100 vigorously-growing 10- to 12-inch plants of each of 24 varieties of flax were dusted with LD_{90}-irradiated spores (in a 1:10 mixture of spores and talc). After incubation for 24 hours in moist chambers, the plants were removed to a greenhouse bench. One week later they were reinoculated, this time with LD_{50}-irradiated spores (in a 1:20 mixture of spores and talc).

An attempt was made to culture each uredium that developed on varieties resistant to the F_1 hybrid urediospores, either on the variety on which it was produced or on Bison. Debility was not as frequent as with ultraviolet-induced mutants (6), but an appreciable number of the X-ray-induced mutants produced insufficient spores for a pathogenicity test. These uredia usually developed an extensive subepidermal stroma,

but spore production was insufficient to break the leaf epidermis. The pathogenicity of the more vigorous mutant cultures was determined on a complete set of differential flax varieties.

Natural mutations.—To reduce the possibilities of recording contaminants as mutants, the study of natural mutations was made in greenhouses entirely separated from those in which other flax rust was grown. Race 251, an X-ray-induced mutant that attacked Dakota, was used. Natural mutants to virulence on Dakota could not be determined with race 251. Natural mutants to virulence on other differential varieties could be distinguished from the X-ray-induced single-gene mutants because Dakota would be attacked and also the variety on which the natural mutation conditioned virulence.

Urediospores of race 251 were screened for natural mutants on one variety at a time during the winter months. Flax was grown in 4½-in. pots, about 20 plants per pot, and inoculated when the seedlings were 4–6 in. tall. Because of limited and variable space, 80–220 pots were sown in each test. One pot of Bison, a rust-susceptible variety, was sown for each 10 pots of the test variety. The plants were inoculated by dusting in incubation chambers with a 1:50 spore-talc mixture of recently harvested urediospores. The number of spores screened for natural mutants was estimated by multiplying the average number of primary uredia that developed on each Bison plant by the number of plants of the test variety.

RESULTS.—*X-ray-induced mutations.*—From 100,000 to 200,000 primary uredia developed on each of the susceptible varieties inoculated with irradiated urediospores. Since at least 1 visible urediospore must have been involved in the initiation of each uredium, the estimate is conservative that each resistant variety screened about 200,000 viable spores for mutations for pathogenicity.

Bombay, Stewart, and Kugine, varieties resistant to the parent races, were not infected by either the irradiated spores or any of the mutants.

No mutant was secured from Abyssinian, B. Golden selection, Bowman, Clay, Kenya, Ottawa 770B, or Pale Blue Crimped, varieties to which the F_1 culture was heterozygous for pathogenicity.

An attempt was made to increase the 154 mutant uredia that developed on Birio, Cass, Dakota, Koto, Leona, Polk, Towner, and Wilden. Table 1 shows the reactions of the rust-differentiating varieties to the 94 cultures that produced sufficient inoculum. With two exceptions, each mutant fell into 1 of 6 pathogenicity patterns differing from the F_1 culture in virulence on a single differential flax variety or a group of varieties. This would be expected following a single "hit" on a gene string. The pathogenicity of the two exceptions was that expected following two "hits" on segments of the gene string at different loci carrying genes for pathogenicity.

Mutants derived from uredia developed on Cass and Dakota differed from the F_1 culture of race 22 × race 1 only in ability to attack each of these varieties. In genetic studies (2) pathogenicity on Cass and Dakota was conditioned by single pairs of independently inherited genes.

TABLE 1.—*Number and pathogenicity of X-ray-induced mutations in an F_1 urediospore culture of race 22 × race 1 of Melampsora lini*

Differential variety	No. of mutant uredia that developed on indicated differential	Reaction [a] of indicated differential [b] variety to											
		F_1 urediospores of race 22 × race 1	Mutants from										
			Dakota	Cass	Koto	Leona	Polk		Towner	Birio	Wilden		
Dakota	22	I	S	I	I	I	I	I	I	I	I	S	
Cass	33	I	I	S	I	I	I	I	I	I	I	I	
Koto	2	I	I	I	S	S	I	S	I	I	I	I	
Abyssinian	0	R	R	R	S	S	R	S	R	R	R	R	
Leona	3	I	I	I	S	S	I	S	I	I	I	I	
Polk	18	I	I	I	I	I	S	S	I	I	I	I	
Marshall	[c]	R	R	R	R	R	S	S	R	R	R	R	
B. Golden selection	[c]	R—	R—	R—	R—	R—	R—	R—	S	R—	R—	R—	
Kenya	0	I	I	I	I	I	I	I	R—	I	I	I	
Pale Blue Crimped	0	R	R	R	R	R	R	R	R—	R	R	R	
Towner	3	I	I	I	I	I	I	I	S	I	I	I	
Birio	24	I	I	I	I	I	I	I	I	S	S	S	
Wilden	49	R	R	R	R	R	R	R	R	S	S	S	
Barnes	[c]	R—	R—	R—	R—	R—	R—	R—	R—	S	S	S	
No. of mutants tested to which differentials had indicated reaction			15	20	2	2	8	1	2	14	29	1	

[a] I = immune; R = resistant; R— = moderately resistant; S = susceptible.

[b] Not shown in first column are differential varieties Ottawa 770B, Bombay, Stewart, Clay, and Bowman, which are resistant, and Akmolinsk, Bison, Burke, Victory A, Ward, and Williston Brown, which are susceptible, to F_1 culture of race 22 × 1 and to all mutants.

[c] Barnes and B. Golden selection had an intermediate reaction to the F_1 culture of race 22 × race 1 and mutants for virulence on these varieties were not conspicuous. Marshall was not inoculated with irradiated urediospores.

The mutants from Koto and Leona were identical in pathogenicity and differed from the F_1 culture by attacking Abyssinian as well as Koto and Leona. Pathogenicity on these three varieties has been inherited as a unit (2).

Both Marshall and Polk were susceptible to the mutants obtained from Polk. In hybridization studies (2), pathogenicity on these varieties has been found to be closely linked but not inherited as a unit. The moderately resistant reaction of Marshall made it less satisfactory for detecting mutant uredia than the highly resistant to immune reaction of Polk, and it was not inoculated with the irradiated urediospores. One of the mutants from Polk also attacked Abyssinian, Koto, and Leona, indicative of a double mutation for pathogenicity.

The mutants from Towner attacked B. Golden selection. Kenya and Pale Blue Crimped were only moderately resistant to the Towner mutants, and, respectively, were immune and highly resistant to the non-irradiated urediospores. Pathogenicity on B. Golden selection, Kenya, and Pale Blue Crimped was found to be inherited as a unit and loosely linked with pathogenicity on Towner (5).

Barnes, Birio, and Wilden were susceptible to the 44 mutant cultures from Birio and Wilden that were tested for pathogenicity. In studies on the inheritance of pathogenicity (2,5), Barnes and Wilden were susceptible to the parent races and to the F_1 and F_2 cultures. Consequently, any linkage relations between the genes conditioning pathogenicity on these two varieties and the other differentials could not be ascertained. This mutation study indicates that the genes for pathogenicity on Birio are closely linked with those for pathogenicity on Barnes and Wilden. One of the mutants from Wilden attacked Dakota besides Barnes, Birio, and Wilden.

In studies on mutations for pathogenicity in the rust fungi, major sources of error are the genetic purity of the spores subjected to irradiation, and any contamination during or following inoculation. Every variant is a potential mutant. New races differing in pathogenicity from previously encountered races are almost certainly mutants.

The F_1 urediospores of race 22 $\times$ race 1 and the 7 mutant races that did not attack Birio differed in pathogenicity from all races previously studied. The mutants attacking Birio, Wilden, and Barnes had the pathogenicity of race 218, an F_2 culture of race 6 $\times$ race 22 (4). Consequently, the possibility exists that the cultures attacking Birio could have been contaminants. This is unlikely, however, for Birio, Wilden, and Barnes were not infected by the non-irradiated spores. Furthermore, the mutant from Wilden that attacked Dakota differed in pathogenicity from any race previously isolated.

Mutation rates.—The limited extent of these experiments and the number of variables involved make an estimate of the relative induced mutation rates an approximation at best. There appeared to be considerable differences in the mutability of the genes for pathogenicity following exposure to X rays.

Although the F_1 urediospores of race 22 $\times$ race 1 were heterozygous for pathogenicity on Bowman, Clay, and Ottawa 770B, no mutants were secured that attacked these varieties. Apparently the genes that condition avirulence on Bowman, Clay, and Ottawa 770B are relatively stable even when subjected to X irradiation.

Mutations to virulence on Abyssinian, Koto, and Leona, and on Towner, occurred with about equal frequency, at a relatively low rate. The status of Kenya and Pale Blue Crimped is not clear. No mutants were screened on them, but both were definitely less resistant to the mutants that attacked Towner than to the F_1 culture of race 22 $\times$ race 1 or to the other mutants.

Mutations to virulence on Dakota were of the same order of frequency as on Polk. They were several times more numerous than mutants attacking Abyssinian, Koto, Leona, and Towner. Mutants attacking Cass and Birio, Wilden, and Barnes were most numerous.

Natural mutations.—Three "natural" mutants, 2 on Birio and 1 on Cass, were secured (Table 2). The cultures from Birio had the pathogenicity of race 259, the X-ray-induced mutant from Wilden that attacked Dakota besides Birio, Wilden, and Barnes. The culture from Cass, designated race 262, differed in pathogenicity from all races previously encountered by attacking Cass in addition to the varieties attacked by race 251. Each of the 3 cultures had the pathogenicity expected if mutation had occurred in a single gene or a single group of closely linked genes in race 251.

The mutations obtained from non-irradiated urediospores were the same as occurred in highest frequency following X irradiation. This suggests that the genes most responsive to the mutagenic effects of irradiation may be the least stable under natural conditions also.

Discussion.—The number of mutations obtained, both X-ray-induced and natural, suggests that mutations to virulence in rusts may be more frequent than reports indicate. Besides the difficulty in distinguishing mutants from contaminants, the techniques often used in studies of physiologic specialization as well as the genetics of host-parasite interaction impede the detection of mutations. To reduce the possibilities of contamination, pathologists often increase rusts on a variety that differentiates them. Consequently, if aviru-

Table 2.—*Natural mutations to virulence in an F_1 urediospore culture of race 22 X race 1 of Melampsora lini*

Differential variety	Minimum number of urediospores screened for mutants	Number of mutants
Birio	200,000	2
Cass	600,000	1
Leona	300,000	0
Polk	900,000	0

lence is dominant, the mutant, unable to infect the variety on which it is being increased, is lost. Unless the culture is heterozygous, a mutation toward wider virulence, conditioned by recessive genes, can be detected only if simultaneous mutations of identical genes occur in both dikaryotic nuclei of a single spore —and then only if the differential variety possesses a single gene for rust reaction.

The present tests explain 2 of the 3 reported patterns of naturally occurring mutations to virulence (8,10,13). The same mutation would be expected to occur repeatedly in a urediospore culture of a race if that culture is heterozygous for a gene of high mutability. Similarly, a number of separate mutations would be expected in a urediospore culture that is heterozygous for a number of genes conditioning pathogenicity, as was the F_1 culture of race 22 $\times$ race 1 of *Melampsora lini* used in this study.

The gradual replacement of one race by a previously unknown race is more difficult to explain. Possibly the mutation to virulence was associated with a mutation to greater vigor, or perhaps a mutation to greater vigor might have influenced the host-parasite interaction balance so as to affect infection type.

The nature of the "mutations" to virulence in the flax rust fungus is not known. They could have resulted either from a change in the gene (point mutation) or from destruction of a segment or even a whole chromosome. All the mutant virulence genes were recessive and functioned in the monokaryon as well as in the homozygous dikaryon. A study of the inheritance of virulence in mutant cultures should make it possible to determine whether the mutation was a gene modification or a deletion.

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STUDIES OF CUCUMBER ANTHRACNOSE[1]

L. V. Busch and J. C. Walker

SUMMARY

Three foreign accessions of cucumber were selected from 135 tested for resistance to *Colletotrichum lagenarium*. The mode of inheritance of resistance in each is described. Penetration of leaves was similar for both resistant and susceptible plants, but cytoplasm and chloroplasts in the former disintegrated more slowly, and beadlike areas developed on the outer surfaces of cell walls.

Barnes and Epps,[2] in the course of screening several hundred foreign accessions of cucumber, found 7 that showed some resistance to anthracnose, incited by *Colletotrichum lagenarium* (Pass.) Ell. & Halst. They reported that resistance in one of the accessions (P.I. 175111) behaved as a monogenic character. Resistance in another accession (P.I. 197087), reported to be of a higher order than that in P.I. 175111, appeared to be controlled by several major genes and possible modifying genes. This paper is a report of a study of the relation of the pathogen to resistant and susceptible host tissue. Inheritance of resistance in the lines used was also studied.

METHODS OF INOCULATION AND RESISTANCE ASSAY.—The isolate of the pathogen used was from a diseased cucumber collected at a market in Madison, Wisconsin. Inoculum consisted of spores from young cultures on potato dextrose or oatmeal agar. Spore suspensions in distilled water were adjusted by hemacytometer to 1½–2 million spores per ml. Inoculated plants, in the second- or third-leaf stage, were placed in a moist chamber for 15–17 hours at 28°C and then removed to a greenhouse bench at the same temperature. Under these conditions a consistent difference in disease reaction was observed between resistant Congo watermelon and susceptible cucumber and watermelon varieties. It is important to note, however, that the differences between resistant and susceptible lines were relative, not absolute. Such differences became less with increases in inoculum load and time in the moist chamber. Symptoms appeared a little more slowly at 22° than at 28°, but eventually became equally severe. The lower temperature was used when plants were inoculated for host-parasite reaction studies.

INHERITANCE OF RESISTANCE IN CUCUMBER LINES STUDIED.—Seed samples of 135 foreign accessions were secured from the United States Department of Agriculture Regional Plant Introduction Station at Ames, Iowa. After resistance assay, 3 lots showing relatively high degrees of resistance were selected for further study: P.I. 163213, P.I. 163217, and P.I. 175111. The last had the same number as the lot that Barnes and Epps[2] reported had monogenic resistance. Crosses were made between plants of each of the 3 lines and those of 1 or more of the following susceptible pickling varieties of cucumber: National Pickling, Ohio MR 17, and Wisconsin SMR 12. In each of 5 F_1 progenies the disease was intermediate in severity between that of the respective parents, but in the cross between Wisconsin SMR 12 × P.I. 175111, the F_1 plants were closer in reaction to that of the resistant parent than was the case in the other 4 crosses. The F_2 progeny from Wisconsin SMR 12 × P.I. 175111 and the progeny from the backcross of F_1 plants with Wisconsin SMR 12 were tested for disease reaction. The segregating F_2 and backcross progenies were separated into a susceptible class resembling in disease severity the susceptible parental line and a resistant class that approached, but was not equal to, the resistant parental line in disease expression. The F_2 progeny were divided into 130 resistant and 57 susceptible plants, while the backcross progeny were divided into 87 resistant and 87 susceptible plants. These ratios were close fits to 3:1 and 1:1, respectively, confirming the report of Barnes and Epps[2] that resistance in P.I. 175111 was inherited as a monogenic character.

Results with F_2 and backcross progenies involving P.I. 163213 as a resistant parent were difficult to interpret. As already indicated, the disease expression of F_1 plants was intermediate between those of the parents. Moreover, the results of individual assays varied considerably. When the plants in F_2 progenies were grouped into classes approaching those of each parent and into an intermediate group, the data conformed to a 1:2:1 ratio, suggesting the possibility of a single gene pair with incomplete dominance. Backcross progenies likewise could be classed roughly into 2 classes, approaching the susceptible parent in 1 case and an intermediate position in the other. It was concluded that more than 1 pair of genes was involved or,

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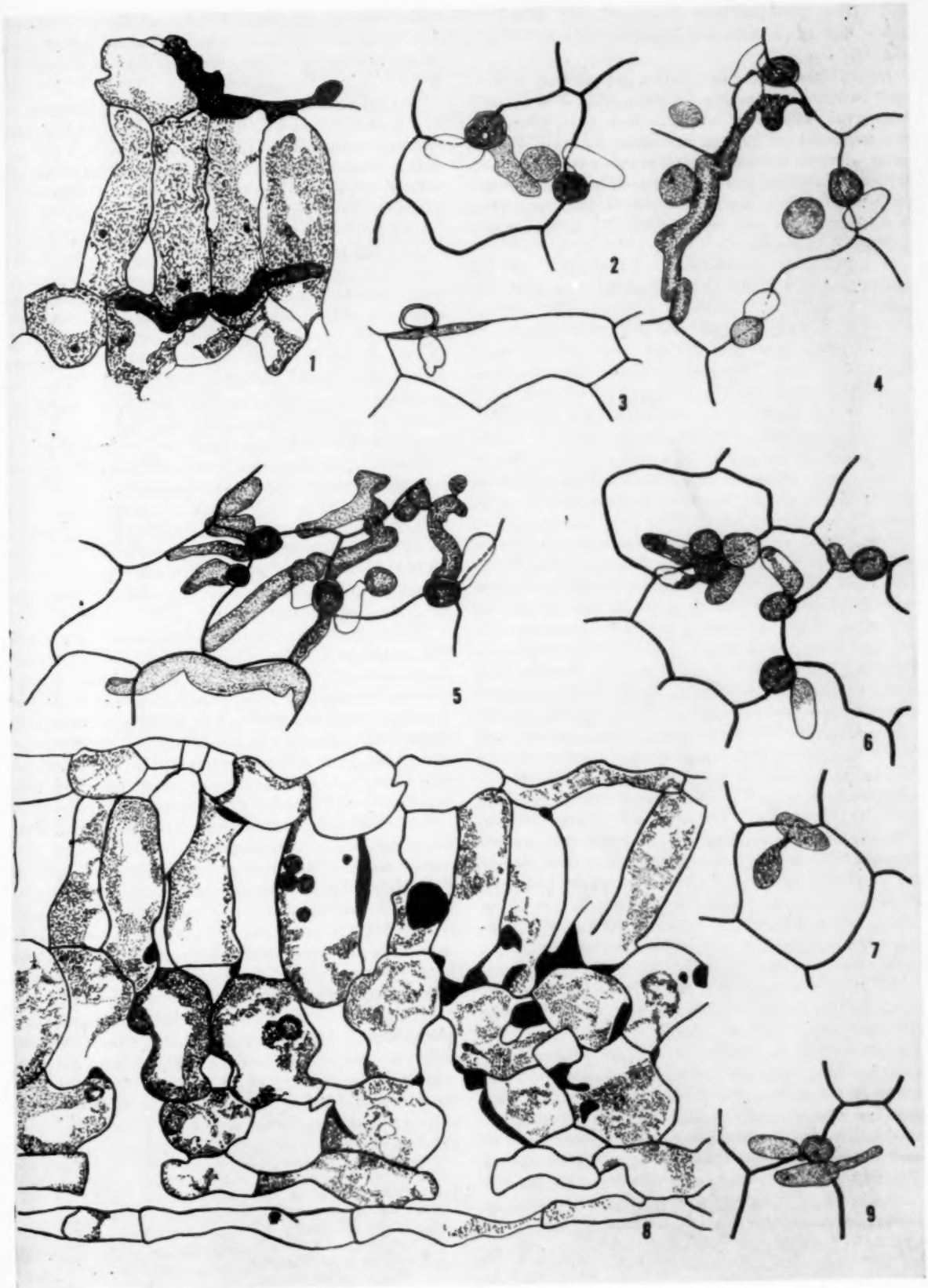
[1] Accepted for publication December 30, 1957.

[2] Barnes, W. C., and W. M. Epps. 1952. Two types of anthracnose resistance in cucumbers. Plant Disease Reptr. 36: 479-480.

FIG. 1-9. Relation of *Colletotrichum lagenarium* to leaf tissue of resistant P.I. 175111 (PI) and susceptible National Pickling (NP) cucumber plants.—Fig. 6, 7. Formation of appressoria on leaves of NP 8 hours after inoculation.—Fig. 2, 3. Penetration of epidermal cells of PI at 68 hours.—Fig. 9. Penetration of NP at 68 hours. Fig. 4, 5. Advance of mycelium in epidermal cells of NP and PI, respectively, at 88 hours.—Fig. 1. Mycelium invading mesophyll cells of NP 74 hours after inoculation.—Fig. 8. Cross section of PI leaf 2 weeks after inoculation; solid black patches represent deep-red-staining areas on outside of cell walls in intercellular spaces and between adjoining cell walls. This reaction in advance of the mycelium was characteristic of resistant leaf tissue.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if 1 major gene pair was concerned, that modifying genes had more influence than in the case of P.I. 175111.

Data obtained from inoculation experiments with F_2 and backcross progenies involving P.I. 163217 were even more difficult to interpret than were data from the progenies of crosses involving P.I. 163213. In many separate tests, over 2,000 plants were inoculated. When disease readings were taken it was not possible to fit the data into any simple genetical interpretation. It was concluded that resistance in P.I. 163217 was a multigenic character.

HOST-PARASITE INTERACTION.—Penetration by the fungus and its subsequent development were first compared in susceptible National Pickling and resistant P.I. 175111. Conidia suspended in distilled water were placed on the upper leaf surface of plants in a moist chamber at 22°C. Sections of leaves were fixed in formalin-propionic-alcohol mixture, dehydrated in a tertiary-butyl-alcohol series, and embedded in paraffin. Material was sectioned on a rotary microtome at 10–12 μ. Conant's quadruple stain was used. Early stages of penetration were observed by clearing leaves in aceto-acid alcohol and mounting in lacto-phenol cotton blue.

Germination occurred promptly and appressoria in some cases were formed within 8 hours (Fig. 6, 7). They were most numerous immediately above the side walls of epidermal cells. Penetration was effected by projection of the infection peg from a germ pore in the appressorial wall attached to the host cuticle, as described by Gardner.[4] The process was usually completed within 68 hours (Fig. 2, 3, 9). Penetration was similar in resistant and susceptible leaves (Fig. 4, 5).

In susceptible leaves the penetration peg invaded the epidermal cell and mycelium proceeded to invade cells of the mesophyll intracellularly (Fig. 1). It was constricted to a very small diameter when passing through cell walls. The invaded cells collapsed promptly and the disintegrated protoplast was stained deep red. Mycelium was prominent and also stained red in the collapsed tissue. The chloroplasts and cytoplasm broke down in uninvaded cells adjacent to, or not far removed from, invaded cells. The mycelium invaded the vascular tissue as well as the mesophyll and spongy parenchyma.

Mycelium was much less prevalent in resistant leaf tissue than in susceptible tissue subjected to the same treatment. The cells in advance of mycelium were characterized by a peculiar reaction of the walls. In fixed sections, bead-like areas stained deep red appeared on the outside of cell walls in the intercellular spaces or between walls of adjacent cells. These areas increased in size in the absence of mycelium and, in some cases, completely filled the intercellular spaces (Fig. 8). There was occasional thickening of the cell walls and such portions of the wall also stained red. The cytoplasm and chloroplasts remained intact much longer in advance of the mycelium than in susceptible tissue.

Limited studies were made of inoculated leaf tissue of P.I. 163213 and P.I. 163217. The host reaction was similar to that of P.I. 175111.

DISCUSSION.—It was shown that resistance, as expressed in the seedling stage, appeared to behave as a dominant monogenic factor in P.I. 175111, as reported by Barnes and Epps.[2] However, it should be pointed out that the plants in the resistant class varied somewhat, not being in all cases equal to the parental line in resistance. Moreover, in the inoculation method of Barnes and Epps,[3] 50,000 spores per ml were used. If a higher concentration had been used, their results would possibly have been different. They suggested that a concentration in excess of 100,000 per ml under greenhouse conditions produced sizeable lesions on resistant plants. It is entirely possible that, although a single gene pair may be responsible for a certain degree of resistance, accumulated modifying genes may play a part in the expression of resistance in P.I. 175111. When the latter was crossed with susceptible cucumbers, the modifying genes may have been so dispersed in F_2 and in backcrosses between the F_1 and susceptible, that only an occasional plant that carried the main gene had sufficient modifying genes to react like the resistant parent.

The study of resistant and susceptible leaves showed that, while penetration occurred equally well in both, the progress of the hyphae was much slower in the resistant tissue. The morphological evidence of host reaction in resistant plants took the form of thickening of the cell walls in advance of the hyphae and red-staining deposits in the intercellular spaces. In general, the chloroplasts and other cytoplasmic material tended to collapse more slowly in resistant tissue.

While cell-wall reactions of resistant tissue suggested the possibility of the formation of a morphological barrier against the pathogen, there was no way of determining by histological methods whether or not this was the primary basis of resistance. The fact that cell contents of resistant tissue reacted more slowly than those of susceptible tissue in advance of the fungus suggested that the latter was less compatible with resistant than with susceptible substrate. The true basis of resistance may well be biochemical, with morphological response a purely secondary reaction. Only P.I. 175111 was studied in detail, but no apparent differences were observed in type of reaction among the 3 resistant lines. However, it was apparent from genetical analysis that resistance in each case was genotypically different.

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[3] Barnes, W. C., and W. M. Epps. 1955. Progress in breeding cucumber resistant to anthracnose and downy mildew. Proc. Amer. Soc. Hort. Sci. 65: 409-415.

[4] Gardner, M. W. 1918. Anthracnose of cucurbits. U. S. Dept. Agr. Bull. 727. 68 p.

THE INFLUENCE OF TEMPERATURE ON DISEASE DEVELOPMENT IN BARLEY INFECTED BY HELMINTHOSPORIUM SATIVUM[1]

R. V. Clark[2] and J. G. Dickson[3]

SUMMARY

The development of spot blotch incited by *Helminthosporium sativum* Pam. King & Bakke, and that of root rot and seedling blight caused by the same fungus, were compared under different temperatures. Six isolates of the fungus and 6 varieties of barley were used. Spore-mycelial suspensions were used to initiate foliage infection. Root rot and seedling blight development was obtained by adding a uniform sand-cornmeal inoculum to the medium in which the barley was planted. The maximum spot blotch development occurred at 28°C, with rapid development at 24° and considerably less at 20° and 16°. Differences in pathogenicity among isolates were noted, and some were nonpathogenic at 20° and 16°. Varietal differences in disease reaction were not pronounced. Root rot and seedling blight developed severely at all temperatures studied; these ranged from 8° to 28°. Maximum development usually occurred at 20°. The isolates differed significantly in pathogenicity, but considerable shifting in ranking occurred within experiments and between experiments. This was true for the response of the barley varieties. Experimental error was large for all root rot and seedling blight experiments.

Helminthosporium sativum Pam. King & Bakke is an important pathogen in most cereal-growing areas and attacks barley, wheat, oats, and many grasses. Luttrell (9), in his investigations of the genus *Helminthosporium*, concluded that this fungus should be referred to as *H. sorokinianum* Sacc. ex Sorokin, and the binomial *H. sativum* reduced to synonymy. Infection may occur at any stage in the growth of the plants; root rot and seedling blight, spot blotch, and head blight are various phases of this disease.

Disease development in plants infected by *H. sativum* is considered to be greatest at warm temperatures or at a temperature unfavorable for the growth of the host. Dosdall (3) found that mycelial penetration of coleoptile and leaf tissues occurred over a range of 12–34°C and that extensive infection and rapid disease development occurred between 22° and 30°. Head blight development was found by Andersen (1) to be most extensive at temperatures of 25–30°C. Observations by Mitra (12) indicated that infection by *H. sativum* in wheat and barley was intensified as the temperature was raised from 20° to 30°. McKinney (10) found in greenhouse tests that root rot developed at all temperatures studied between 8° and 35°, but that infection was much less toward the extremes than near the middle of this range. The optimum soil temperature for disease development was found to be 28° with Hanna and Hannchen barleys and Marquis wheat, and 32° for Harvest Queen wheat. McKinney noted that soil moisture influenced the temperature at which maximum disease development occurred. In field studies, Sallans (13) and Greaney (4) noted that seedling blight development was more severe when wheat was sown late in the spring than when sown early. Low soil temperatures were associated with low disease incidence and high yield. In contrast, Hynes (6) found that certain isolates of *H. sativum* produced severe disease on wheat seedlings at relatively low temperatures. Mead (11) noted that temperature and moisture were closely associated in disease development by this fungus and severe blighting occurred at temperatures of 10–30°, provided there was sufficient moisture for good seedling growth.

Because of the conflicting data on the influence of temperature on disease development by *H. sativum*, it was felt that a re-evaluation of the temperature requirements of this pathogen would reveal some useful information. This paper summarizes some of the data obtained in this study.

MATERIALS AND METHODS.—Experiments were conducted in the greenhouse under controlled conditions. Barley seedlings to be used for the study of spot blotch development at different temperatures were grown in white quartz sand in 6-in. clay pots, 6 varieties per pot, 10 kernels per variety, and 3 replicates per experiment. The pots were placed on greenhouse benches in controlled-temperature houses. Planting was done first at 16°C, 7 days later at 20°, 10 days later at 24°, and 13 days later at 28°. Fourteen days after the final planting the seedlings at all 4 temperatures were inoculated with uniform spore-mycelial suspensions of the fungus prepared from wheat kernel cultures. The suspensions were applied with an atomizer used at approximately 10-lb. air pressure. Eight days after inoculation, spot blotch development was recorded by estimating the percentage of the total leaf area covered by lesions and necrosis.

Inoculum used for the development of root rot and seedling blight was produced by growing *H. sativum* on a sterilized nutrient containing sand, cornmeal and

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salt by the method used by Ludwig, Clark, Julien, and Robinson (8). One part cornmeal by weight was added to 19 parts quartz sand; these were mixed thoroughly, dispensed into flasks, and moistened with a nutrient solution containing the inorganic salts of a Czapek's medium (7) plus 5 per cent peptone. In all experiments, 10-day-old inoculum was mixed with white quartz sand at the rate of 5 per cent by weight, and this mixture was used as the planting medium. Before being mixed with the sand, the sand-cornmeal inoculum was passed through a screen of $\frac{1}{8}$-in. mesh to break up any lumps. Following a thorough mixing of the inoculum and the sand, equal amounts by volume of the mixture were placed in the planting containers. First, the containers were partly filled and the contents uniformly and lightly tamped. Then 10 kernels were placed in the center of each container, if only 1 variety per container was used, or placed in well-separated clumps of 10 kernels each when more than 1 variety per container was used. Finally, the kernels were covered to a uniform depth of 1 in. with the remainder of the planting medium and lightly tamped. A uniform moisture content for optimum growth of the plants was maintained by adding $\frac{1}{2}$ N Hoagland's solution (5). The barley seed used in all experiments was surface sterilized by soaking the kernels for 30 seconds in a 1:1 mixture of 40 per cent ethyl alcohol and 2.5 per cent sodium hypochlorite (Klenzade). It was washed later in sterile water and then dried. Only undamaged kernels of uniform size were used for planting.

Barley seedlings for the comparison of root rot development at various temperatures were grown in 2 different ways. In most cases, the mixture of inoculum and sand was dispensed into 10-oz. paper cups with a hole in the bottom for drainage. One variety of barley was planted per cup. The cups were placed in greenhouse flats in the various temperature houses when the same soil and air temperatures were used. When a soil colder than air temperature was employed, the cups were placed in aluminum trays that in turn were placed in the Wisconsin temperature tanks (2). The temperature throughout the pans was kept constant by placing moistened greenhouse sand around the cups. In 2 experiments, planting was done in 6-in. porcelain crocks; in these, 6 varieties were sown per crock. The crocks were placed directly in the controlled-temperature tanks. A piece of glass tubing was placed in an upright position in each crock to facilitate watering.

After 2 weeks of growth, notes on root rot and seedling blight development were recorded. The seedlings were removed from the planting containers and washed free of inoculum and sand. The measurement of the mean height of each clump of seedlings, excluding root system, was recorded. In addition, the seedlings were examined individually and classified for degree of coleoptile discoloration and lesioning, stunting, and blighting. A disease index was calculated from these data based on the following assigned values: 0, no discoloration and lesioning; 1, slight discoloration and lesioning; 2, moderate discoloration and lesioning; 3, severe discoloration and lesioning with stunting; 4, blighted seedlings. By application of McKinney's formula (10), a discoloration or disease index was obtained. Since growth was fastest at the high temperatures, all results were recorded as the differences between the inoculated seedlings and the control seedlings for each temperature.

RESULTS.—*The influence of temperature on spot blotch development.*—Six monoconidial isolates of *H. sativum* and 6 varieties of barley were used at 4 temperatures. The average results of 2 experiments are presented graphically in Fig. 1. Spot blotch development was greatest at 28°C. in both experiments. Disease development decreased as the temperature was lowered; with certain isolates, no disease developed at temperatures of 20° and 16°. There was a statistically significant difference in the over-all response of the isolates, and the differences were consistent throughout the various temperatures studied. Isolates 5B and 13C were highly pathogenic, whereas isolates 10C and 6C were much less virulent. The latter 2 isolates were nonpathogenic at the low temperatures on the majority of the varieties. There were only small differences among the barley varieties in their response to the isolates of the fungus; however, the variety Olli was consistently the most susceptible.

The influence of temperature on the development of root rot and seedling blight.—The same 6 isolates of the fungus and 6 varieties of barley used in the previous tests were employed in these studies. An experiment was set up to determine the effect of the same soil and air temperature on disease development. The seedlings were grown in a mixture of white quartz sand and inoculum, and this mixture was placed in paper cups in greenhouse flats. The experiment was run in 2 parts, with 3 isolates of the pathogen plus a control each time and 3 replicates for each isolate at each temperature.

The data on the development of root rot and seed-

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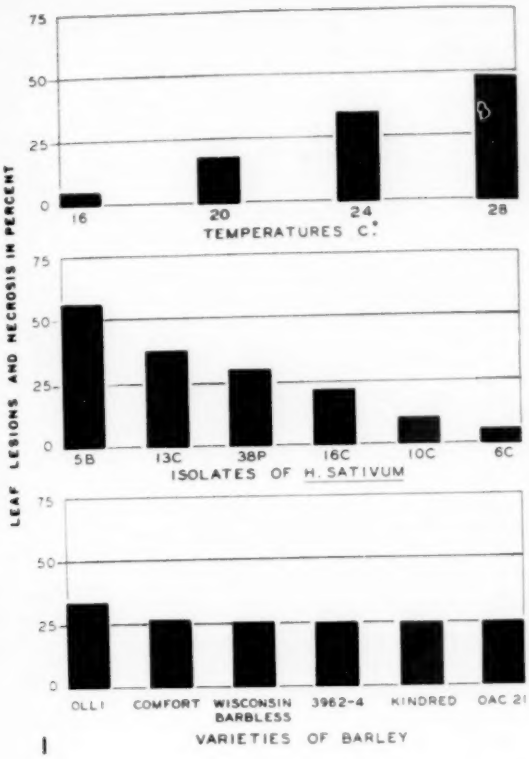
FIG. 1, 2, 4, 6.—Disease development in barley infected by *Helminthosporium sativum* at various temperatures.—Fig. 1. Average development of spot blotch at 4 temperatures on 6 varieties inoculated with each of 6 isolates of the fungus.—Fig. 2. Development of root rot and seedling blight at 4 temperatures on 6 varieties inoculated with each of 6 isolates of the fungus and grown in constant temperature houses for 14 days. Data based on differences between inoculated and control seedlings.—Fig. 4. Development of root rot and seedling blight at soil temperatures held 4°C lower than the air temperature for each of 4 temperature comparisons in which 6 isolates of the fungus and 6 varieties grown in controlled temperature tanks for 14 days were used. Data based on differences between inoculated and control seedlings.—Fig. 6. Influence of alternating temperatures on development of root rot and seedling blight incited by 3 isolates of the fungus on 3 varieties grown in constant temperature houses for 14 days. Data based on differences between inoculated and control seedlings.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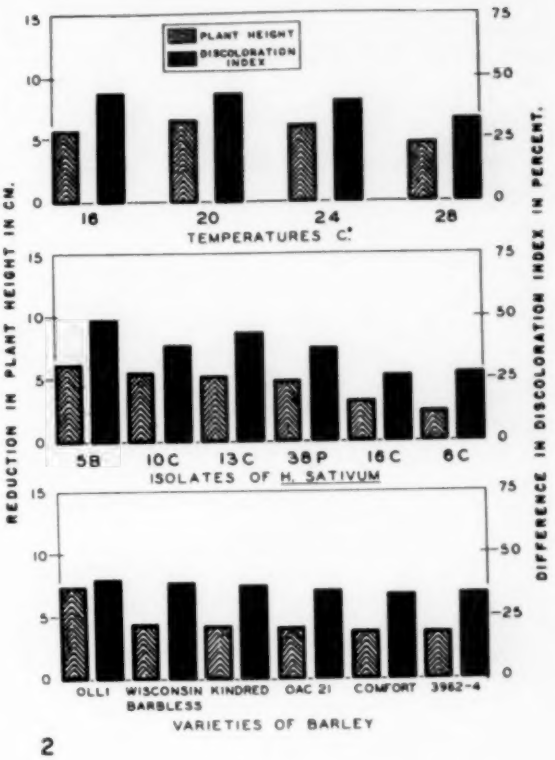
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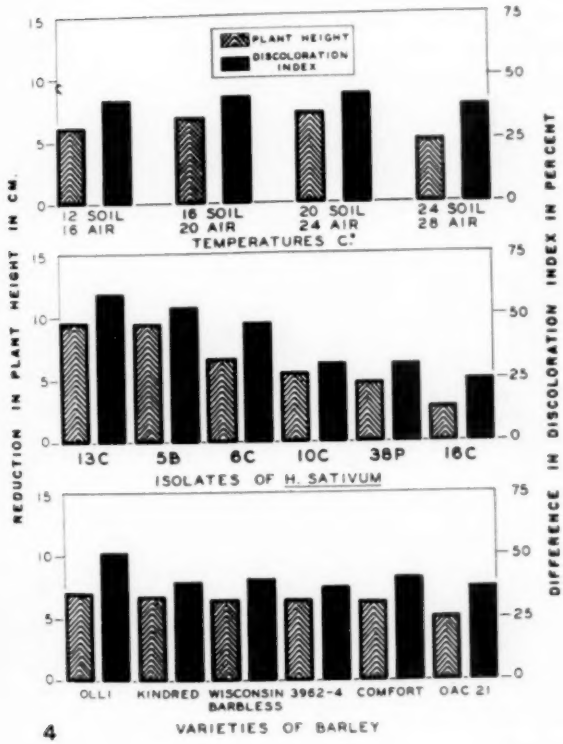
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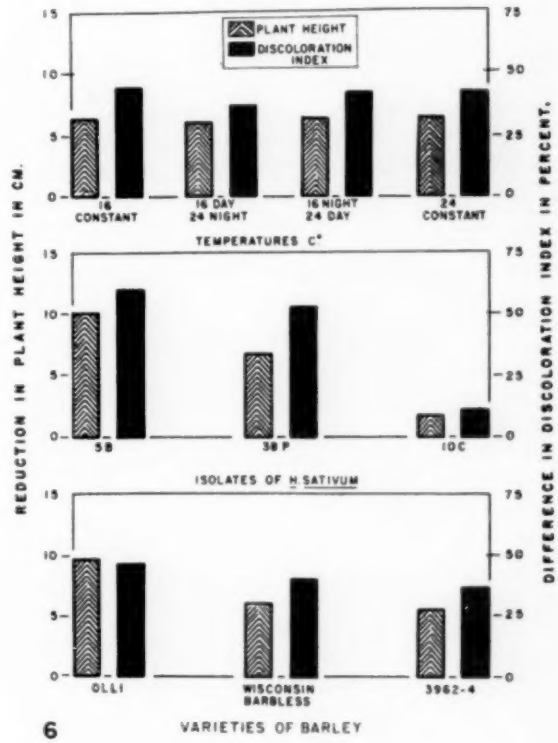
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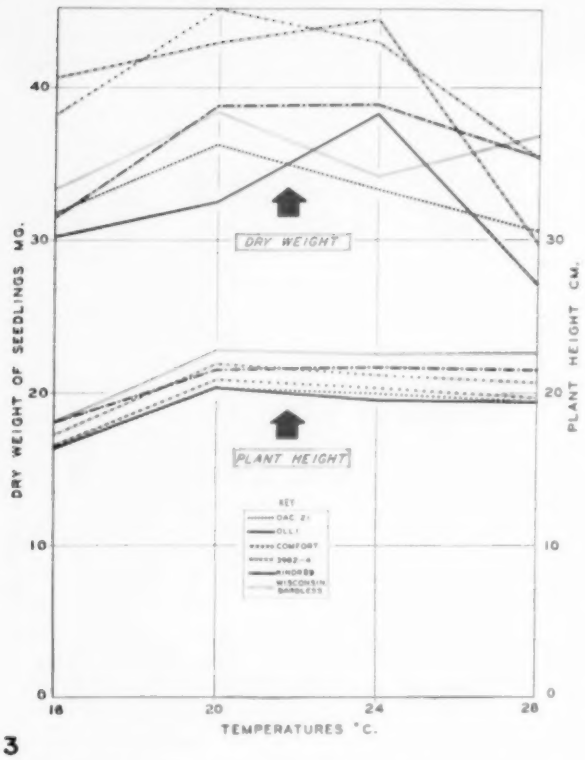

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FIG. 3—Influence of 4 temperatures on the development of 6 noninoculated barley varieties grown in the greenhouse for 14 days.

ling blight at the different temperatures are summarized in Fig. 2. Disease development was severe at the 4 temperatures studied; however a statistical analysis of the data showed that there was a highly significant difference in response to the various temperatures. The maximum disease development occurred at 20°C. This was most obvious when the height of the seedlings was used as a criterion and less so when the discoloration indices were used. The isolates of *H. sativum* were significantly different in pathogenicity, ranging from highly virulent to weakly virulent. The barley varieties were significantly different in response to the isolates and also to temperatures. The variety Olli was the most susceptible to root rot development, but the other 5 varieties differed little in this respect. Varietal reactions at the various temperatures were inconsistent; for this reason, considerable variability in the data was evident; also, there was some inconsistency in the action of the various isolates.

The previous experiment was repeated with the exception that the 6 isolates of *H. sativum* were compared simultaneously at each temperature. Planting was done in 6-in. porcelain crocks, and the 6 varieties of barley were included in each crock. These were placed in controlled-temperature tanks. As sufficient tank space was not available to replicate the experiment, it was repeated 3 times and the 3 experiments were considered as replicates in the analysis of the data. The over-all results were similar to that of the previous experiment in that root rot development was severe at the 4 temperatures; however, there was less difference than before in disease development at the various temperatures, and none of the differences in the height data were statistically significant. The isolates of the fungus again differed in pathogenicity, and the varieties of barley responded differently. Data on temperature effects again were variable; in some cases, the ranking of the isolates and of the varieties at the different temperatures did not agree with those of the previous experiment. In this experiment, the dry weight of each clump of seedlings was obtained in an effort to secure an accurate measure of disease development. The weight figures for each group of seedlings were calculated on a single-plant basis, and disease development was obtained by comparing the inoculated seedlings with the controls. The data on dry weight per plant, as a measure of disease response, did not agree with the results based on seedling height and discoloration indices. The dry weight data were similar to the 2 other sets of data with respect to the overall influence of temperature on disease development, but the dry weight data for the reaction of the isolates and the response of the barley varieties was quite different from that of the height data and discoloration indices. The dry-weight measurements, however, did provide a comparison of the growth of the various noninoculated barley varieties at the 4 temperatures (Fig. 3). The optimum temperature range for the growth of the barley varieties was 20–24°C. The 2 sets of data were somewhat similar at 16°, 20°, and 24°, but the height data overemphasized plant development at 28°.

In another experiment, comparisons were made at 4

FIG. 5.—Influence of an 8°C soil temperature and a 16° air temperature on the development of root rot and seedling blight incited by 6 isolates of *H. sativum* on O.A.C. 21 barley seedlings grown in controlled temperature tanks for 32 days. From left to right, control, and isolates 16C, 10C, 38P, 6C, 5B, and 13C.

temperature combinations in which the soil temperature was held 4°C lower than the air temperature. The 6 isolates of the fungus and 6 varieties of barley used in earlier experiments were used. The seedlings were grown in the planting medium in paper cups, which were placed in trays in the temperature tanks. The test was set up in 2 parts, with 3 isolates compared at a time; there were 3 replicates in each case. Root rot and seedling blight development was severe at the 4 temperature combinations, with an apparent optimum at a soil temperature of 20° and an air temperature of 24° (Fig. 4). These results correspond in general with the temperature response in the previous experiments. The development of root rot and seedling blight at the various temperatures was significantly different when both types of data were analyzed statistically but the differences in the height data were greater than those in the discoloration data. The isolates differed significantly in pathogenicity and there was little change in ranking at the various temperatures. The barley varieties also differed significantly in degree of infection. However, their reaction at the different temperatures was not consistent. For example, at the 2 lowest temperatures the variety 3962-4 was the most susceptible, while at the highest temperature this variety was the most resistant, when the plant height data were considered. The varietal response was not so variable at the different temperatures when the discoloration index data were used as a criterion.

It was apparent from this experiment (Fig. 4) that a soil temperature of 12°C and an air temperature of 16° did not limit the development of root rot and seedling blight. For this reason, a test was set up with a soil temperature of approximately 8° and an air temperature of 16° to determine the extent of disease development at lower temperatures. The 8° soil temperature was maintained by means of refrigerated temperature tanks. The isolates of *H. sativum* and varieties of barley used previously were used. Planting was done in paper cups placed in aluminum trays; there were 3 replicates of each treatment. Because of the slow rate of growth at this low temperature, disease development notes were not recorded until 32 days after planting. Even at a soil temperature of 8°, the disease development by certain isolates (13C and 5B) was severe (Fig. 5). This test was repeated with 6-in. porcelain crocks rather than paper cups used as planting containers. Results were similar to those of the previous test, and the ranking for the pathogenicity of the isolates was the same for the 2 tests. There was, however, considerable variation between the 2 experiments in the response of the varieties.

The influence of alternating temperatures on root rot and seedling blight development was investigated in a preliminary manner. Seedlings were grown at a 16°C constant temperature, an alternating 16° day (9 hours) and 24° night, an alternating 16° night (15 hours) and 24° day, and a 24° constant temperature. Three of the isolates of *H. sativum* and 3 of the barley varieties used previously were employed. Planting was done in paper cups, which were placed in greenhouse flats. The data indicated that the alternating temperatures did not change materially the disease response from that obtained with constant temperatures (Fig. 6). There was no statistically significant difference between the effects of any 2 of the several temperature combinations. The isolates differed significantly in pathogenicity and were consistent through the various temperatures; however, the varieties were again variable in their response.

DISCUSSION.—The results obtained in the present study indicated that the spot blotch phase of the disease caused by *H. sativum* responds differently to a temperature series than does the root rot and seedling blight phase caused by the same fungus. Spot blotch development was most intensive at a temperature of 28°; symptoms developed quite quickly at temperatures of 24° and 28°, somewhat slower at 20°, and considerably slower at 16°. The more pathogenic isolates caused wilting of the foliage at temperatures of 24° and 28°. These results agree with those of Dosdall (3) for disease development on coleoptile and leaf tissues and with those of Andersen (1) for the development of head blight. Root rot and seedling blight developed over a wider temperature range than did spot blotch. Severe root rot developed at temperatures from 8° to 28°, and maximum development usually occurred at 20°. Similar results were reported by Mead (11) in greenhouse and field studies; however, McKinney (10), from greenhouse studies, and Sallans (13) and Greaney (4), from field studies, concluded that cool temperatures are unfavorable for disease development. The different response to temperature by the spot blotch phase and the root rot and seedling blight phase of this disease might be explained by the findings of Ludwig, Clark, Julien, and Robinson (8), who concluded that a toxic principle is involved in the development of the root rot and seedling blight phase of the disease incited by this fungus. In the present studies, possibly the mass of inoculum in close association with the seedlings in the soil produced a sufficient concentration of toxin for severe root rot development at the cool temperatures. This may also explain the conflicting reports in the literature.

The data obtained on the influence of temperature on the development of spot blotch were consistent between experiments and within experiments, but this was not the case with the data on the development of root rot and seedling blight. The over-all influence of temperature was similar in the various experiments, but the action of the isolates of *H. sativum* in inciting root rot and the response of the barley varieties to infection were quite variable both within experiments and between experiments. The results suggested that most of the variability was due to the inconsistent reaction of the barley varieties at the various temperatures. In certain experiments, the several interactions involved were significant, and in some cases the differences between replicates were significant. This variability was due partly to variations in environ-

mental conditions in the greenhouse in which the tests were conducted. In most experiments, the data on plant height showed a good correlation with the discoloration indices, but there was less variability in the discoloration indices. It was felt, however, that the height data were the more reliable measure of disease development, since considerable discoloration occurred on the coleoptiles of the seedlings in the controls.

When the dry weights of the seedlings, both roots and tops, were compared with data on height of the seedlings and discoloration of the coleoptiles, the dry-weight data did not agree with the 2 other sets of data. The dry weights of the tops alone would probably have been the most reliable measure of disease development. The extent of decomposition of the barley kernels was variable, and it was difficult to remove all the sand and inoculum from the root system; both factors added to the variability of the weights. The dry-weight data based on the whole plants did show that the optimum temperature for the growth of the barley varieties ranged from 20° to 24°C, which agrees with the findings of McKinney (10).

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INFLUENCE OF BACTERIAL SOFT ROT, DEPTH TO WATER TABLE, SOURCE OF NITROGEN, AND SOIL FUMIGATION ON PRODUCTION OF LETTUCE IN THE EVERGLADES [1]

J. P. Winfree, R. S. Cox and D. S. Harrison [2]

SUMMARY

In the Everglades, control of the bacterial soft rots of lettuce in the field apparently depends upon cumulative effect of several practices: use of foliar pesticides, water control, and careful fertilization. The soft rots can be controlled in transit by streptomycin. Development of bacterial soft rot was enhanced by a higher water table; whereas growth and quality of lettuce were impaired. Soil acidity was decreased at the higher water tables; mineralization of soil nitrogen was apparently less rapid. Fumigation with chloropicrin provided no control of the soft rots, but it did exert a decided influence on the kinetics of ammonification and nitrification in the organic soil. Ammonium nitrogen increased initially at the expense of nitrate nitrogen. This was followed within 40–70 days, depending on the depth to water table, by replacement of ammonium nitrogen by nitrate nitrogen.

A paradoxical situation has been pointed out (2) pertaining to the apparent suitability of the Everglades for lettuce production, and the actual minor role the crop plays in the agricultural economy of the area. Factors that appear to favor lettuce production are: (1) the fertile organic soils; (2) the controlled water supply and subsurface irrigation; (3) the cool weather with low rainfall that prevails during the period from December through April; and (4) the proximity to eastern markets.

In spite of these apparent desirable factors yields are quite erratic and lettuce grown in the Everglades has a reputation at market terminals of inferior keeping quality.

Several diseases appear to contribute to erratic yields as well as to perishableness of lettuce in transit. Diseases affecting yield include downy mildew (caused by *Bremia lactucae* Regal), bacterial soft rots (caused by *Erwinia* sp. and perhaps *Pseudomonas* sp.), bottom rot (caused by *Rhizoctonia solani* Kuhn), and a disease of unknown origin described as rib breakdown (1). Transit diseases include the soft rots and rib breakdown. An effective control program for downy mildew was devised (2) and progress has been made on the control of soft rot in transit (1). Attempts at control of bottom rot have not been too successful.

Observations reveal that the water table is not carefully controlled and, because of insufficient information on nutrition of lettuce, proper fertilization practices probably are not followed. The results of work during the period 1955-57, inclusively, pertaining to control of soft rot, and to the effect of certain cultural practices on disease incidence and on growth and quality of lettuce are presented in this paper.

THE DISEASE.—*Symptomatology.*—Bacterial soft rot occurs in the field in two phases: jelly butt and slime head (1). The disease is first evidenced by a sudden wilting of the lower leaves (Fig. 1,A). Twenty-four hours later, the outer leaves are completely wilted (Fig. 1,B) in a manner suggestive of sclerotiniose. Sliming of the head frequently follows. Death of the plant occurs within 48 hours after initial symptoms.

Close inspection reveals that wilting of the leaves is preceded by a vascular discoloration of the stem (Fig. 2,A) which gradually extends into the midrib, thence to the lateral veins of the leaf (Fig. 2,D,E,F). It is not until later that the entire butt breaks down (Fig. 2,B,C,D) and the head slimes. This suggests that jelly butt and slime head may be secondary manifestations, and the disease, a vascular wilt.

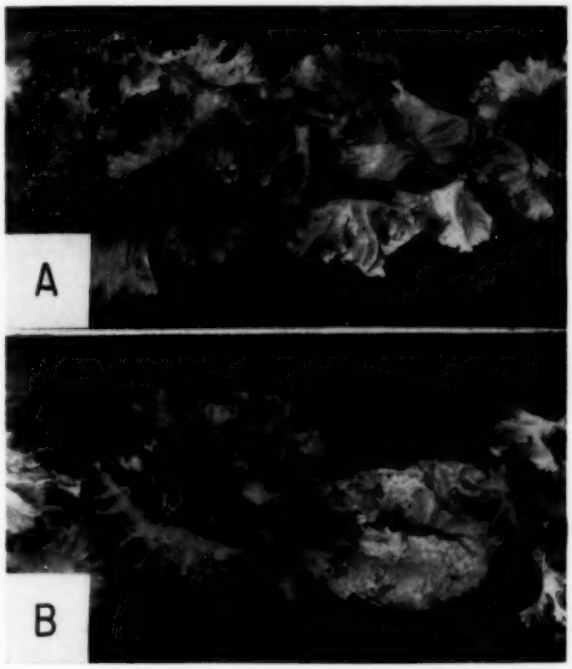

FIG. 1.—Stages of development of bacterial soft rot of lettuce in the field. A) initial wilting. B) 24 hours later.

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Bacteria of the Erwinia soft rot type (1) are associated with all phases of the disease. Dr. Grover Sowell, Gulf Coast Experiment Station, Bradenton, Florida, in unpublished work, has isolated a *Pseudomonas* sp. in addition to an *Erwinia* sp. from jelly butt material.

Control.—The bacterial soft rot pathogen(s) was successfully introduced into test plots in the fall of 1955 by means of transplants from an infested field. As shown by subsequent disease development, inoculum increased rapidly. Streptomycin sulfate sprays (100–200 ppm) applied throughout the season resulted in less soft rot than was present in unsprayed plots. The material was phytotoxic, however, at both concentrations causing a marked chlorosis.

During 1956 streptomycin sulfate was again tested. Concentration was reduced to 25 and 50 ppm, and the material was applied only during the latter half of the growing season. No appreciable control was effected under these conditions nor was the material phytotoxic. Bacterial soft rot incidence was virtually 100 per cent regardless of treatment. The failure of streptomycin sulfate when applied during the latter part of the season leads to the suggestion that initial infection occurs during the early part of the season. If this is true streptomycin should be applied earlier than it was in this test.

In a test in the spring of 1957, there was significantly less disease where TBCS (tribasic copper sulfate) plus Agrimycin-100 (50 ppm streptomycin sulfate) was applied throughout the season than in unsprayed plots (Table 1). Streptomycin sulfate at 25 ppm plus TBCS was ineffective.

Growers frequently produce a marketable crop only to lose it from rots in transit. Greatest losses are due to jelly butt and slime head. Tests were undertaken to determine the effect of streptomycin applied as a dip or painted on the butts during packaging operations. Results from representative tests are shown in Table 2. A high degree of control of jelly butt and slime head resulted from dipping in streptomycin sulfate solutions at concentrations as low as 10 ppm (Table 2). Jelly butt also was controlled effectively

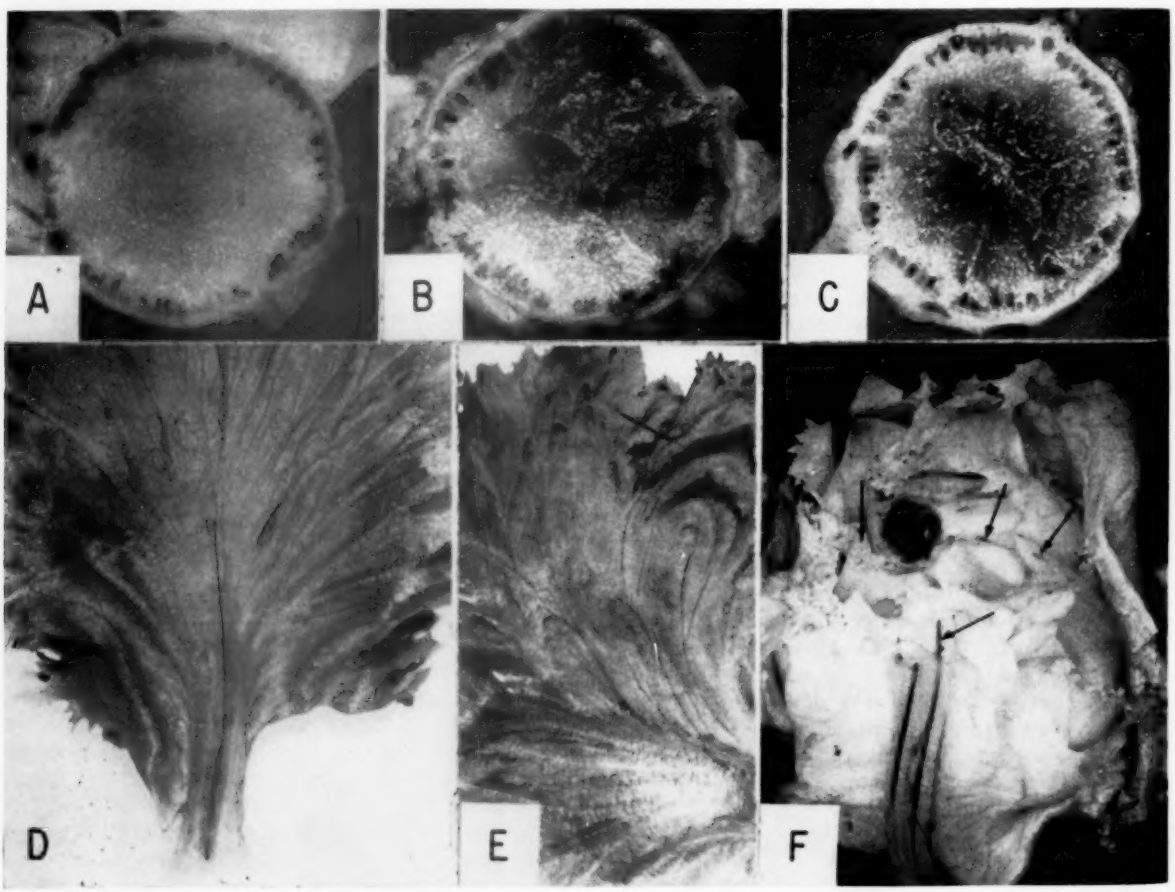

FIG. 2.—Stages of development of bacterial soft rot on stems and leaves of lettuce. A) Vascular discoloration in stem. B) Early stage of jelly butt. C) Advanced stage of jelly butt. D) Vascular discoloration in midrib. E) Vascular discoloration of lateral veins and initial wilting of leaf (note arrow). F) Advanced stages of jelly butt and vascular discoloration in stem. Note that discoloration of the midrib originates at juncture of stem and leaf (arrows in cutaway portion of head).

TABLE 1.—*Development of bacterial soft rot in field-grown lettuce sprayed with various materials alone and in recombination, 1957*[a]

Materials[b]	Amount in 100 gal. lb.	Amount in 100 gal. ppm[c]	Diseased Percentage of Heads: Jelly Butt (wilted)	Diseased Percentage of Heads: Vascular infection (not wilted)	Diseased Percentage of Heads: Total
None			60	36	96
TBCS	4 lb.		52	36	88
TBCS Agrimycin-100	4 lb.	25 ppm	43	38	81
TBCS Agrimycin-100	4 lb.	50 ppm	38	26	64
Parzate	2 lb.		57	33	90
Parzate Agrimycin-100	2 lb.	25 ppm	48	28	76
Thylate	1½ lb.		51	30	81
Spergon	2 lb.		43	31	74
Parzate Thylate	1½ lb.		50	31	81
L.S.D. .05			20	N.S.	25
.01			N.S.	N.S.	30

[a] Plots consisted of single 50-foot rows, 4 replications, in randomized blocks. Materials were applied with a self-propelled spraying rig on a 5-day schedule starting as soon as germination was completed and continuing until a week prior to harvest.

[b] Chemical composition and per cent active ingredient of materials listed as follows: TBCS, basic copper sulfate (53 per cent metallic copper); Agrimycin-100 (15 per cent streptomycin sulfate and 1.5 oxytetracycline 4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide; Parzate (65 per cent zineb, zinc ethylenebis [dithiocarbamate]); Thylate (65 per cent thiram, bis (dimethylthiocarbamoyl) disulfide); Spergon (50 per cent chloranil, tetrachloro-*p*-benzoquinone).

[c] Parts per million of streptomycin sulfate.

when the butts were painted with a solution of Agrimycin-100 containing 250 ppm streptomycin sulfate.

Combined effects of water table and source of nitrogen, and of water table and soil fumigation—1) *Materials and methods.*—Three 25 × 25 ft. plots, with 18-, 24- and 30-in. water tables, were constructed as follows. An area of land on the Everglades station was selected where the elevation was 18 in. above that of the adjacent canal. The necessary amount of surface soil was added with a bulldozer, packed and leveled to raise the elevation of one plot 6 in., and that of the other 12 in. The 18-, 24-, and 30-in. pots will be referred to hereafter as plateaus I, II, and III, respectively.

Three consecutive experiments were on these plateaus, one each in the spring of 1956, fall of 1956 and spring of 1957. The Iceberg No. 118 variety was used in the first experiment and the Iceberg No. A-36 variety in the second and third. Both varieties are highly susceptible to soft rot. Each plateau comprised 16

TABLE 2.—*Development of bacterial soft rot in lettuce given various post-harvest treatments*

Test	Materials	Treatment	ppm of streptomycin sulfate	Percentage of heads with bacterial soft rot[a]: Jelly butt	Percentage of heads with bacterial soft rot[a]: slimehead
1	None	10-minute dip	—	40	6
	Agrimycin-100		500	10	0
	DO		100	10	0
	DO		200	8	0
	L.S.D. .05			10	
2	None	Momentary dip	—		84
	Agrimycin-100		10		12
	DO		25		7
	DO		50		4
	Phytomycin[b]		50		6
	Streptomycin sulfate		50		9
	L.S.D. .05				24
	.01				40
3	Check	Painted butts	—	58	
	Agrimycin		250	4	

[a] Out of a sample size of 200 heads per treatment.

[b] A formulation containing 20 per cent streptomycin nitrate.

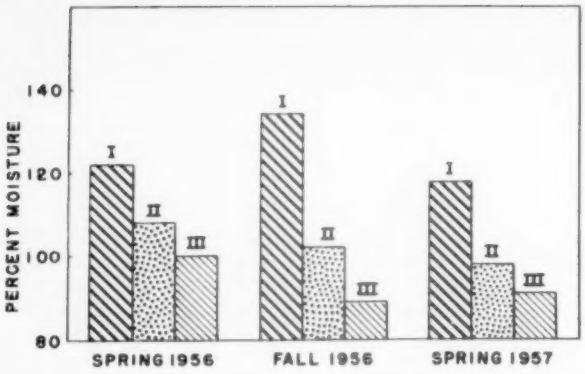

FIG. 3.—Average soil moisture content for the plateaus. I, 18-in. water table; II, 24-in. water table; III, 30-in. water table.

rows 18 in. apart. Plants were spaced 14 in. apart in the row. Thus, there were about 22 plants to the row and 352 to the plot. In the first experiment, no treatments were superimposed on the plateaus; in the second, they were used as blocks in which nitrogen treatments, as sidedressings, were replicated; in the third, one half of each plateau was fumigated with chloropicrin (trichloronitromethane).

Soil samples (top 6 in.) for moisture determinations were taken at weekly intervals during all 3 experiments.

2) *Experimental results.*—a) *Water Table.*—Average moisture content of the soil on the 3 plateaus for the 3 experiments is shown in Fig. 3. It varied inversely with depth to the water table.

Although soil pH varied with season and weather conditions (rainfall primarily), it consistently was inversely related to the depth of the water table. The range in plateaus I, II, and III was pH 5.5–5.8, pH 5.2–5.6, and pH 5.0–5.4, respectively. Ground water in this area characteristically contains large quantities of calcium and magnesium and has a reaction of pH 7 or higher.

Root development of the lettuce also was better where the water table was low than where it was high.

A nitrogen response was indicated in the first experiment where protein-N contents of the plants were for plateaus I, II, and III, respectively, 1.3, 1.6, and 1.6 per cent (mean for 3 harvests), and again in the second experiment where protein-N contents were 1.4, 1.6 and 1.6 per cent (mean of 4 treatments).

In the first experiment, it was observed that plants on plateaus II and III (24- and 30-in. water table) had a deeper green color and superior flavor than did plants on plateau I (18-in. water table). The same trends in respect to these 2 quality factors occurred in the second and third experiments as well; however, the trends were not as evident in tests 2 and 3.

The plants were inoculated with the bacterial soft rot organisms by scattering over the plots soil obtained from an area of land known to be infested. The organisms were successfully established, but the incidence of disease was less than 5 per cent during the first (spring, 1956) season. By the end of the second season (fall, 1956), incidence in plateau I reached 96 per cent (Fig. 4). It was progressively less (61 and 47 per cent) in plateaus II and III. This suggested that increase of inoculum in the soil is favored by a high water table. In the third test (spring, 1957), disease incidence in plateaus I, II, and III was, respectively, 100, 100, and 90 per cent. This suggested that the intensive cropping increased the inoculum potential.

b) *Water table and source of nitrogen.*—In the second test (fall, 1956), 18 days before the lettuce was harvested, plots on the plateaus received the following treatments: 1) no N, 2) $NaNO_3$, 3) NH_4NO_3, and 4) $(NH_4)_2SO_4$. The materials were applied as side dressings at the rate of 100 lb. of N per acre.

There was markedly less soft rot on plateaus II and III, though not on I, where $(NH_4)_2SO_4$ was applied than where other forms of N (or no N) were applied (Fig. 4).

c) *Water table and soil fumigation.*—In the third test (spring, 1957) one half of each plateau was fumigated with chloropicrin applied with a hand injector at the rate of 43 gal. to the acre. Fumes were confined with a water seal. The plots were seeded 10 days after treating. Samples of soil to a 6-in. depth were obtained weekly with a soil tube. They were analyzed immediately or after storage in a deep-freeze. Ammonium-N and nitrate-N determinations were performed as follows. A 100-g sub-sample of soil was extracted, first in an Erlenmeyer flask and then on a Buchner funnel, with a total of 400 ml of *N* NaCl. Ammonium-N was determined by distilling with NaOH, collecting in boric acid, and titrating with HCl, as in the usual Kjeldahl method for N. Nitrate-N was determined in the same solution after reduction with Devarda's alloy following the distillation of ammonia (3). Percentage moisture was determined on separate sub-samples of soil so that concentrations of N could be expressed on a dry-weight basis.

Ammonium-N accumulated in the treated portions

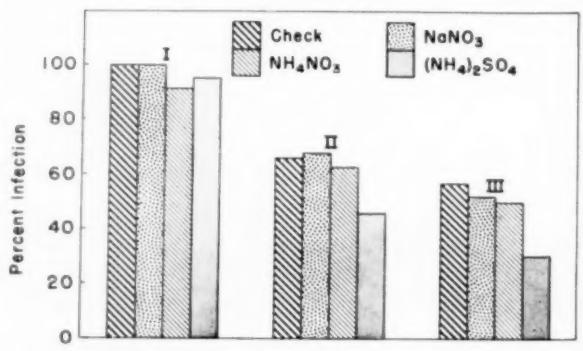

FIG. 4.—Bacterial soft rot of lettuce (expressed as percentage of heads infected) as influenced by depth of water table and source of nitrogen. I, 18-in. water table; II, 24-in. water table; III, 30-in water table.

of plateaus I, II, and III for about 30, 50, and 65 days, respectively (Fig. 5,A,B,C). After reaching maxima of 100–125 ppm, concentrations of ammonium-N decreased rather rapidly as nitrate-N increased. Equal concentrations of the 2 forms of N were reached at about 55, 65, and 75 days for plateaus I, II, and III, respectively. Differences in concentrations between the fumigated and the untreated portions of the 3 plateaus are shown for ammonium—N (Fig. 6,A) and for nitrate-N (Fig. 6,B). These graphs indicate more clearly than Fig. 5 the combined effects of the fumigation treatments and the water table on the length of time that ammonium-N accumulated, the magnitude of the accumulation, and the rate at which it was replaced by nitrate-N.

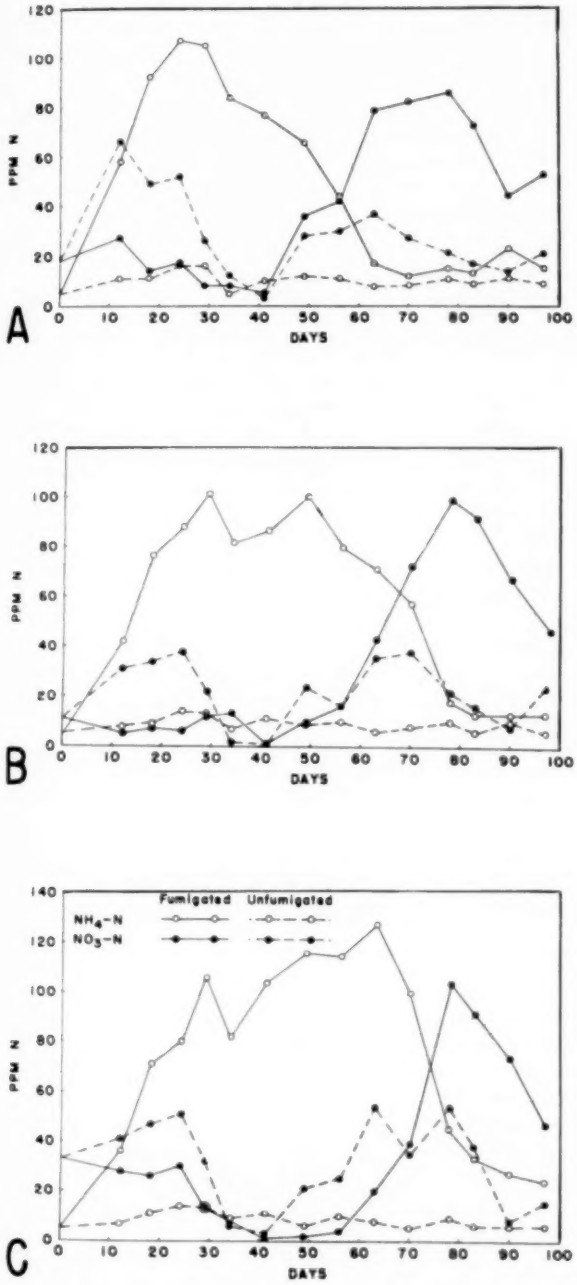

FIG. 5—Ammonification and nitrification in the soil as influenced by depth of water table and fumigation with chloropicrin. A) 18-in. water table. B) 24-in. water table. C) 30-in. water table.

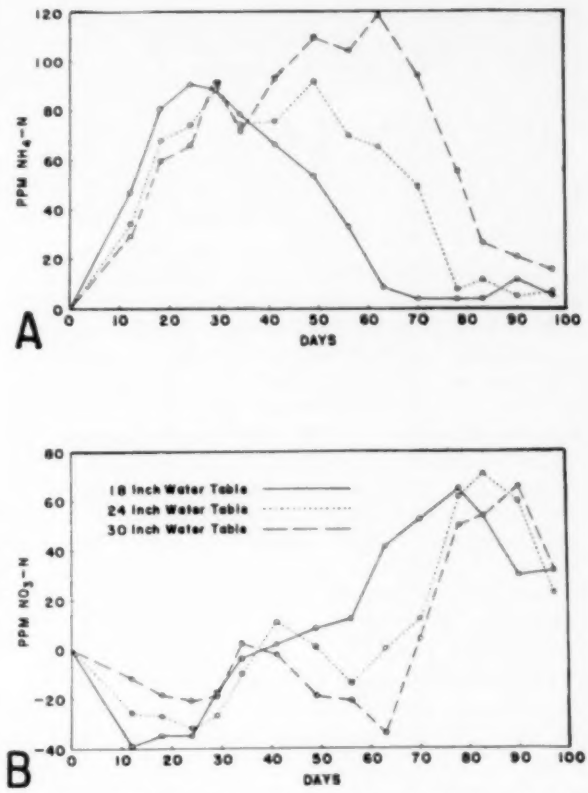

FIG. 6—Differences in concentrations of A) ammonium-N and B) nitrate-N in the soil resulting from fumigation with chloropicrin at three depths of water table. Concentrations in untreated portions have been subtracted from the concentrations in the fumigated portions, hence the negative values for nitrate-N during the first 50-70 days.

Soft rot appeared earlier in the treated than in the untreated portions. Ultimate infection was 100 per cent in all plots except the untreated portion of plateau III (30-in. water table). Even there, 83 per cent of the heads were diseased. It is apparent that chloropicrin is ineffective against the soft rot pathogen(s) involved in this work.

DISCUSSION.—Although none of the methods tested in this work provided adequate control of bacterial soft rot, the results indicated certain possibilities. Streptomycin sprays provided some control. Also, it was shown that the depth of the water table influenced incidence of the disease, probably through its effect on soil moisture. Incidence of soft rot in each of 3 lettuce crops, and the increase in incidence from season to season were influenced by the water table. The increase was less rapid at the lower water tables. The seasonal increases in disease indicate that inoculum

built up rapidly in the soil. Since this may have resulted in part from the very intensive cropping, crop rotations may retard the build-up of inoculum in the soil.

Whereas attempted individual control measures were inadequate, the degrees of control obtained by various practices seem to be cumulative. Incorporation of all of them into a concerted program might result in practical control of soft rot.

The striking responses in transformation of nitrogen that followed chloropicrin fumigation of the soil would appear to be due to the influence of fumigation on ammonification and nitrification through its action on the organisms involved. Several effects have been suggested (4, 5, 6) as factors involved in accumulation of ammonia following "partial sterilization" of soil. Probably the most important action of the chloropicrin in the organic soil was that against the nitrifiers resulting in the accumulation of ammonium-N. That nitrification was virtually stopped for a time was also evidenced by the period following accumulation of ammonium-N, which was characterized by a direct, apparently stochiometric, replacement of the ammonium-N by nitrate-N.

The ineffectiveness of chloropicrin against bacterial soft rot evidently did not result from the recovery of the causal bacteria following an initial partial kill. In view of the likelihood that infection occurs early in the life of the host plant, it appears more probable that chloropicrin is not lethal to the soft rot bacteria as they exist in the organic soil. Since disease developed more rapidly in fumigated plots than in untreated ones, it might be implied that treatment with chloropicrin actually resulted in favorable conditions for infection, perhaps by upsetting or shifting the microbial population in favor of the soft rot organism.

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EXTRACELLULAR PECTIC AND CELLULOLYTIC ENZYMES OF CLADOSPORIUM CUCUMERINUM [1]

Akhtar Husain and Saul Rich

SUMMARY

Cladosporium cucumerinum Ell. & Arth. produced relatively large amounts of extracellular PG (polygalacturonase) when grown on dextrose, but little or none on citrus pectin. No PME (pectin methylesterase) was detected. The organism also produced extracellular cellulase on filter paper, wood cellulose, or carboxymethylcellulose. The cellulase is adaptive, for only a small amount was produced on dextrose. Juice from a scab-resistant strain of cucumber did not inhibit the enzyme *in vitro*. Of several fungicides and metallic ions studied, the mercuric ion inhibited cellulase, and a few fungicides partially inhibited PG. It was concluded that tissues in the infected host are disintegrated by cellulase and PG produced by the fungus.

Cladosporium cucumerinum Ell. & Arth., the cause of scab of cucumber, is an important pathogen in northwestern Europe and some parts of the United States and Canada. Various workers have studied the host-parasite relationship in the disease. Bond (2) reported that, after penetration through the stomata, the fungus became inter- and intracellular and caused the death of cells without any evidence of extensive enzymatic activity. Behr (1) found that penetration occurred directly through the epidermal cell wall, and that cavities were produced in the infected tissues of the fruit by cytolysis. Pierson and Walker (10), comparing susceptible and resistant cucumber varieties in a detailed histological study, found that the pathogen usually penetrates directly through epidermal cells. Cells were killed in advance of actual invasion of tissue by the pathogen. Complete lysis of cell walls and formation of large cavities were observed in parenchymatous tissue of susceptible hosts. Cellulose of the cell walls was also altered in susceptible tissue. These observations suggested that enzyme action was an important part of the pathogenic processes of this disease.

On the basis of these observations, a study was undertaken of the secretion of pectic and cellulolytic enzymes by *C. cucumerinum* and the relation of these enzymes to disease resistance. The effect of fungicides on the enzymes was also determined.

[1] Accepted for publication January 16, 1958.

MATERIALS AND METHODS.—A modified Richard's solution containing 2 per cent dextrose and 0.1 per cent yeast extract was used as the basal nutrient medium for preparing culture filtrates for enzyme assays. Other carbon sources used were filter-paper pulp, wood cellulose (Solka Floc, Brown Co.), carboxymethylcellulose (CMC 30 low; low viscosity and low substitution; Hercules Powder Co.), and citrus pectin (Sunkist Growers, Inc.). Erlenmeyer flasks (500-ml capacity) containing 100 ml of the liquid medium were seeded with 2 ml of a spore suspension of the fungus and placed on a wrist-action shaker at room temperature (about 25°C). Some of the cultures were grown without shaking. A fungus culture 4–7 days old was filtered through paper and the filtrate centrifuged 20 minutes at 3000 rpm to remove spores. The clear supernatant was used as the enzyme sample.

PME (pectin methylesterase) activity was measured by the continuous titration method of Kertesz (6), using as the substrate 1 per cent solution of citrus pectin adjusted from pH 5.0 to 7.0. PG (polygalacturonase) assays were carried out by measuring the loss in viscosity of the substrate and the increase in reducing groups. Viscosity was estimated in size-300 Ostwald Fenske viscometers at 32°C (±.01). A 1.2 per cent solution of sodium polypectate (Sunkist Growers, Inc.), or citrus pectin containing citric acid-sodium hydroxide buffer at pH 5.5, was used as substrate. Increase in reducing groups was measured by the Willstätter-Schudel hypoiodite method as modified by Jansen and MacDonnell (4).

Cellulase activity was determined by measuring the loss in viscosity of the substrate and the increase in reducing sugar. Viscosity was measured by a method similar to that for PG, except that the substrate was 0.5 per cent solution of carboxymethylcellulose (CMC 70 high) containing citric acid-sodium hydroxide buffer at pH 5.5. CMC 70 high is a high-viscosity highly-substituted material from Hercules Powder Company. To determine the reducing sugar, 1 ml of culture filtrate was added to 9 ml of about 0.5 per cent solution of CMC 30 low that contained 0.05 M acetate

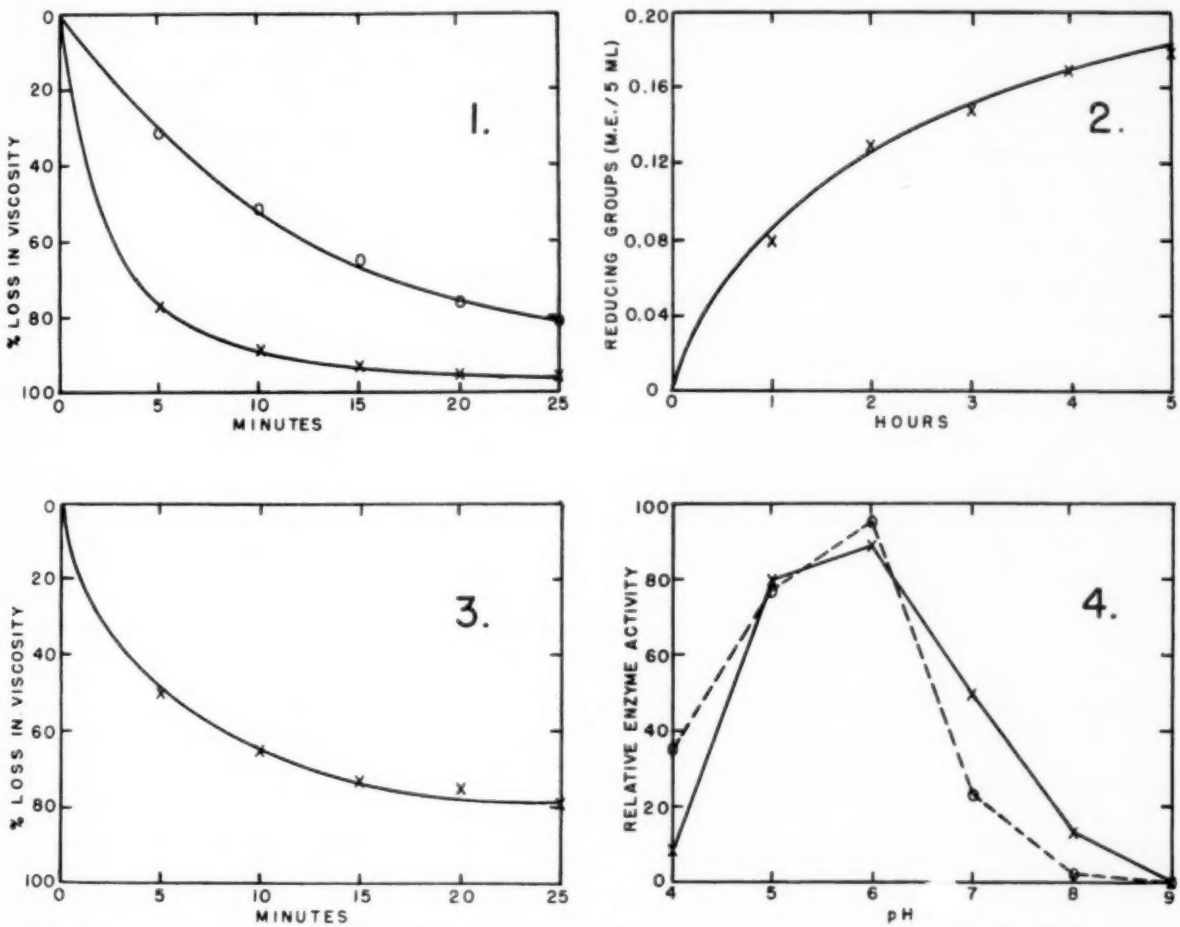

FIG. 1–4.—Observations with culture filtrates of *Cladosporium cucumerinum*.—1. Loss in viscosity of pectin (o) and sodium polypectate (x) caused by diluted (1:1) culture filtrate.—2. Milliequivalents (M.E.) of reducing groups produced by 5 ml of a reaction mixture of 0.5% sodium polypectate with a culture filtrate.—3. Loss in viscosity of a 0.5% solution of carboxymethylcellulose (CMC 70 high) caused by a diluted (1:1) culture filtrate.—4. Effect of pH on the PG (o) and cellulase (x) activities of a culture filtrate.

TABLE 1.—*Effect of fungicides on the cellulase and the polygalacturonase of C. cucumerinum*

Compounds	Per cent inhibition	
	PG	Cellulase
10^{-3}M Hg++	nt[a]	99.5
10^{-3}M Mn++	nt	0
10^{-3}M Zn++	nt	0
10^{-3}M Cu++	nt	0
10^{-3}M Fe+++	nt	0
10^{-3}M Ag+	nt	0
0.1% Ferbam	40.5	0
0.1% Ziram	50.4	0
0.1% Dichlone	44.1	0
0.1% Pyridinethione (Na salt)	37.0	0
0.1% Nabam	54.9	0
0.1% Zineb	0	0
0.1% Maneb	0	0
0.1% Cycloheximide	0	0
0.1% Captan	0	0
0.1% Spergon	0	0
0.1% 8-quinolinol sulfate	0	0
0.1% 8-quinolinol benzoate	0	0

[a] The symbol "nt" means "not tested."

buffer at pH 5.5. Final concentration of the substrate in the reaction mixture was 0.5 per cent. Test tubes containing the reaction mixture were incubated 2 hours in a water bath of 35°C, and 1-ml samples were withdrawn before and after. Reducing sugar was determined by Nelson's modification (8) of the micromethod of Somogyi, and enzyme activity was expressed in terms of the increase in reducing sugar per ml of reaction mixture in 2 hours. Each test was carried out in triplicate. A boiled culture filtrate and a 0.25 per cent solution of a commercial cellulase preparation (Enzyme 19AP; Rohm and Haas Co.) were used as controls.

RESULTS.—*Enzymes produced by C. Cucumerinum.*—Culture filtrates obtained from dextrose- or pectin-containing media gave negative tests for PME; filtrates obtained from a dextrose medium showed strong PG activity. Even a diluted (1:1) culture filtrate rapidly decreased the viscosity of sodium polypectate. The enzyme was found to be significantly less active on citrus pectin (Fig. 1). When 10 ml of culture filtrate was added to 90 ml of sodium polypectate, a significant increase in reducing groups was detected after incubating 1 hour (Fig. 2). Significantly more enzyme was produced in still culture than in shake cultures, although the latter produced considerably more mycelial mat. When 1 per cent pectin was the carbon source, the organism made excellent growth but produced little or no PG, either in shake or still cultures. This differs from results with other pectolytic fungi, where pectic substances generally increase enzyme production (3).

The organism also produced a cellulase when CMC 30 low, CMC 70 high, wood cellulose, or filter-paper pulp was used as the source of carbon. Thus, culture filtrates from all cellulose-containing media caused rapid loss in viscosity of CMC solution, indicating the presence of cellulase (Fig. 3). Reducing sugar was produced when the enzyme was incubated 2 hours with CMC solution. The enzyme activity was far less than for a 0.25 per cent solution of Enzyme 19AP. One ml of the mixture of culture filtrate and substrate gave 0.08 mg of glucose, whereas the comparative figure for 0.25 per cent Enzyme 19AP was 0.56.

Very weak cellulase activity was detected in filtrates from dextrose-containing media. To investigate the ability of the fungus to degrade a native source of cellulase, filter-paper strips were suspended in incubating mineral nutrient containing ammonium nitrate as the nitrogen source and 0.01 per cent yeast extract. The fungus made good growth on the strips, but they weakened only slightly in 2 weeks; complete breakdown did not occur.

To determine the effect of temperature on enzyme production, the cultures were incubated at a constant 23° or 28°C. One per cent CMC 30 low was the source of carbon. Each experiment, with 4 replications, was repeated once. After 10 days of culture growth, the cellulase activity of the culture filtrates and the mycelial dry weights were determined at each temperature. Enzyme activity was measured as the reciprocal of the time for 50 per cent loss in viscosity. The average mycelial weight at 28°C was 127 times as great as the weight at 23°C; but the relative cellulase activity in the filtrates was 28 times as great from cultures grown at 23°C as from those grown at 28°C. These results are similar to those obtained with *C. herbarum* by Reese, Siu, and Levinson (13).

Properties of the enzymes.—The PG from *C. cucumerinum* was allowed to act on sodium polypectate, and the hydrolytic products were examined chromatographically. Ten ml of dialyzed culture filtrate was added to 90 ml of 1 per cent sodium polypectate adjusted to pH 5.5. The mixture was incubated under aseptic conditions in a water bath at 30°C. Aliquots of 10 ml were removed at intervals of 8, 16, 32, and 48 hours, and the hydrolytic products determined on descending paper chromatograms. Small amounts of different samples were applied on Whatman No. 1 paper strips, along with a 1 per cent solution of D-galacturonic acid, and the hydrolyzate obtained from the action of Pectinol-100D (Rohm and Haas Co.) on sodium polypectate. A sample of heat-inactivated enzyme mixed with substrate was used as a control. The chromatograms were allowed to run for 20–24 hours, using a 4:1:5 mixture of butanol, acetic acid, and water as the solvent. The dried chromatogram was sprayed with aniline hydrogen phthalate and heated at 105°C for 5–10 minutes (9). After 8 hours of incubation, a significant amount of galacturonic acid was detected, along with 3 more intermediate substances of higher molecular size.

To determine the influence of pH on the activity of PG and cellulase, solutions of sodium polypectate and CMC 70 high were adjusted to different pH values —with McIlvain's citrate buffer up to pH 7.0, and Sorenson's borate buffer above pH 7.0. The activity of each enzyme was measured in terms of the recipro-

cal of the time for a 50 per cent loss in the viscosity of the substrate. The pectic enzyme was active at pH 4.0–8.0, with the optimum at around pH 6.0. Activity below pH 4.0 (Fig. 4) could not be measured, because the substrate solidified at lower pH values. The cellulase was active at pH 4.0–8.0, with the optimum at pH 6.0 (Fig. 4).

Inhibition of enzyme activity by fungicides.—To find out whether fungicides inhibit the enzymes of *C. cucumerinum*, several fungicides and heavy metals were tested for their ability to inhibit PG and cellulase *in vitro*. Unless indicated otherwise, a 0.01 per cent solution of the fungicides was used. The enzyme was allowed to remain in contact with the inhibitor for 1 hour prior to its addition to the substrate. Final concentration of the inhibitor in the reaction mixture was 0.06 per cent. Enzyme activities were determined by the viscometric methods previously discussed. The results are given in Table 1.

The following fungicides partially inhibited PG activity: ferbam (ferric dimethyldithiocarbamate), ziram (zinc dimethyldithiocarbamate), nabam (disodium ethylenebis[dithiocarbamate]), dichlone (2,3-dichloro-1,4-naphthoquinone), and 2-pyridinethiol-1-oxide. The following fungicides did not inhibit PG activity: zineb (zinc ethylenebis-[dithiocarbamate]), maneb (manganous ethylenebis[dithiocarbamate]), cycloheximide (3-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]-glutarimide), captan (*N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide), chloranil (tetrachloro-*p*-benzoquinone), 8-quinolinol sulfate, and 8-quinolinol benzoate. All of the fungicides tried as inhibitors of PG were also tested against the cellulase. None were inhibitory. In addition, the following metallic ions were tested against cellulase: Hg^{++}, Mn^{++}, Cu^{++}, Fe^{+++}, and Ag^{+}. Only Hg^{++} was inhibitory.

Apparently the cellulase of *C. cucumerinum* differs from those of *Myrothecium verrucaria* and *Trichoderma viride*. Thus nabam and zineb, which Reese and Mandels (11) reported as inhibitors, were found to be completely ineffective against the cellulase of *C. cucumerinum*. This enzyme also differs from the cellulase of *Aspergillus oryzae*, which is inhibited by 8-quinolinol sulfate (5). Neither nabam nor zineb inhibits the cellulase of *C. cucumerinum*, even at very high concentrations.

The juice of resistant and susceptible varieties of cucumber was added to culture filtrate containing PG and cellulase to determine whether resistance is associated with a host-contained inhibition of either of these enzymes. Seedlings 6–8 in. tall of the resistant variety SMR-12 and the susceptible variety Marketer were frozen and ground. The expressed juice was filtered through 2 layers of cheesecloth and centrifuged 20 minutes at 10,000 rpm. Equal parts of the clear supernatant and the culture filtrate were mixed and allowed to stand 1 hour before addition of the substrate. A mixture of water and culture filtrate was used as a control. One ml of the enzyme-juice mixture was added to 5 ml of the CMC 70 high solution, and the activity determined viscosimetrically. Juice from neither variety affected cellulase activity.

Since the raw centrifuged juice caused the sodium polypectate to gel, PG activity could not be measured. Consequently the juice was dialyzed 24 hours against distilled water and added to culture filtrate, as indicated above. The dialyzed juice of neither variety affected PG activity when sodium polypectate was the substrate.

Discussion.—Behr (1) and Pierson and Walker (10) observed that considerable disorganization occurs in tissues infected by *C. cucumerinum*. The present studies provide an explanation for the mechanism of the dissolution of cell walls by this pathogen. The organism, by producing both cellulolytic and pectic enzymes extracellularly, is capable of degrading the two most important constituents of the host cell walls. The production of cellulase provides an explanation for the formation of cavities in the infected tissue. The action of pectic enzyme alone would separate the cells, but complete dissolution of cell walls cannot occur unless the cellulose of the cell walls is degraded. The degradation of both the pectic and cellulosic constituents would cause complete disappearance of cells and the formation of lysogenous cavities. Pierson and Walker also showed that cellulose of cell walls was altered in the infected tissue of a susceptible variety of cucumber, providing further evidence that a cellulolytic enzyme produced by the fungus is active *in vivo*. The swelling of cell walls observed by Pierson and Walker also may be due to the action of cellulase produced by the fungus. Evidence has been provided by Marsh (7) and Reese and Gilligan (12) that cellulases from different fungi increase the alkali swelling of cotton fibers, and Marsh has also shown that a similar change occurs in cotton attacked by cellulolytic organisms. A similar phenomenon may be involved in a swelling observed by Pierson and Walker in the cell walls of infected tissue of susceptible cucumber variety.

It has been shown by Walker (14) and Pierson and Walker (10) that susceptible cucumber tissue becomes resistant at higher temperatures (27°C). This resistance may be related to decreased cellulase production at higher temperature, as observed in the present studies.

The results also indicate that no inhibitor of pectic enzyme is present in resistant seedlings of cucumber. However, it may be possible that the inhibitor either is firmly bound to constituents of cell walls or protoplasm, is dialyzable, or is produced in response to infection.

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EFFECT OF ALFALFA MOSAIC AND BEAN YELLOW MOSAIC VIRUSES ON FLOWERING AND SEED PRODUCTION OF LADINO WHITE CLOVER [1]

K. W. Kreitlow and O. J. Hunt [2]

SUMMARY

In the greenhouse, alfalfa and bean yellow mosaic viruses reduced flowering in a clone of Ladino white clover by 31.7 per cent. In field plots, the viruses in 2 clones reduced flowering by 20.3 and 43.9 per cent, respectively, and seed yield by 29.2 and 53.7 per cent, respectively. There were no differences between virus-infected and healthy plants in viability of seed harvested or percentage of abnormal seed. The viruses were not transmitted through seeds.

It has been shown (7) that virus infection can reduce the yield of Ladino white clover (*Trifolium repens* L.) 23–55 per cent. Also, virus infection stunts most plants and results in less stoloniferous spread than normal. Observation of healthy and virus-infected plants of clones growing in both greenhouse and field suggested that similar drastic effects might occur in flowering and seed production. Also investigated were the effect of virus infection on viability of seed and the possibility of seed transmission of the viruses. An abstract of this work has been published (8).

Materials and methods.—Methods used for obtaining and culturing healthy and virus-infected clones of Ladino white clover under greenhouse and field conditions were described earlier (7). Two clones previously studied were used in this investigation. They were inoculated with a mixture of alfalfa mosaic and bean yellow mosaic viruses. In the greenhouse test, 32 healthy rooted cuttings of clone R63 were transplanted into each of 10 alternate compartments in a sand-nutrient-culture bench, and an equal number of virus-infected rooted cuttings were transplanted into each of the 10 remaining compartments. Plants in each compartment were supplied with a complete nutrient solution. The number of flower heads produced on healthy and virus-infected plants was recorded. No seed was produced in the greenhouse, since Ladino white clover is self-sterile. In the field test, healthy and virus-infected rooted cuttings of clones R63 and R92 were established in triplicate in 3 × 10-ft. plots, each containing 120 plants spaced about 6 in. apart. Data on comparative seed production in the naturally pollinated field plots were obtained by collecting all of the ripened heads in 3 1-sq.-ft. areas within each plot. After the heads in each sample were counted, the seeds were threshed by hand. Germination tests were conducted on 600 scarified seed from virus-infected plants and 600 seed from healthy plants of each clone.

To determine whether the viruses were seed transmitted, 1000 seeds from healthy and virus-infected plants of each clone were sown in separate rows of 100 seeds in flats of steamed soil and the seedlings observed for development of virus symptoms. Juice from the leaves of seedlings in each row was tested on Bountiful bean (*Phaseolus vulgaris* L.) plants to determine whether virus was present in the juice.

Results.—*Greenhouse tests.*—When maximum flowering occurred, 50 days after transplanting, 1322 flower

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The authors are indebted to Dr. J. S. Tidd, of Associated Seed Growers, Inc., New Haven, Conn., for generously supplying seed of Bountiful beans.
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TABLE 1.—*Average number of flower heads and seed yield in healthy and virus-infected clonal plots of Ladino white clover, 1956*

Clone		No. of flower heads	Seed yield (g per sq. ft.)
63	Healthy	156.0	3.31
	Virus-infected	87.5	1.53
	% reduction from virus infection	43.9	53.7
92	Healthy	99.5	2.22
	Virus-infected	79.4	1.57
	% reduction from virus infection	20.3	29.2

heads had developed on the 320 healthy plants, and 902 flower heads on the same number of virus-infected plants. This represents an average of 4.13 heads per healthy plant and 2.81 heads per infected plant, or a reduction in flower production of 31.7 per cent.

FIELD TESTS.—The average number of flower heads produced, with the seed yield in grams per sq. ft., in healthy and virus-infected clonal plots of Ladino white clover, is presented in Table 1. Percentage reduction in numbers of flower heads as a result of virus infection was 43.9 for clone R63 and 20.3 for clone R92. The percentage reduction in seed yield from virus infection was comparable: 53.7 for clone R63 and 29.2 for clone R92. These results verify the differences obtained in the greenhouse and emphasize the substantial reduction in flowering and seed production that may occur among plants infected with these viruses.

SEED GERMINATION TESTS.—There was no appreciable difference in the viability of seed harvested from virus-infected and healthy plants. Seeds from healthy plants of clones R63 and R92 germinated 89.3 and 78.8 per cent, respectively, and those from virus-infected plants, 90.8 and 77.2 per cent, respectively. Microscopic examination showed no difference between healthy and virus-infected plants in per cent of abnormal seeds.

Virus transmission through seeds.—No virus symptom indicative of seed transmission was observed in any of the seedlings developed from seeds from virus-infected plants. No symptoms of virus infection developed in Bountiful beans that were inoculated with juice from leaves harvested from each test row of clover seedlings. No symptoms suggesting virus transmission through the seed were observed in thousands of white clover seedlings of diverse origin that were grown in the greenhouse for other studies. This suggests that seed transmission of these two viruses does not occur in white clover.

DISCUSSION.—No extensive literature exists on the effect of virus infection on flowering and seed production in true clovers. Dickson and McRostie (3) harvested seed from 22 virus-infected and healthy plants of red clover selected at random in the field. Each healthy plant averaged a yield of 484.8 seeds, whereas each virus-infected plant yielded only 65 seeds.

In addition to reduced flowering and seed yield, virus infection in some species results in light, shriveled seed. This occurs mostly in those species in which viruses are transmitted through the seeds. Seed transmission of viruses seems to be more characteristic for leguminous plants than for others. Even so, there is much variability among different hosts and in regard to different viruses. Dickson (2) reported mosaic symptoms developed in a low percentage of red clover seedlings derived from virus-infected parents. The virus or viruses involved were not identified. A suspected seed-borne virus disease of subterranean clover (*Trifolium subterraneum* L.) (1), later identified as bean yellow mosaic virus (6), was reported in Australia. Another, as yet unidentified, aphid-transmitted virus disease of subterranean clover in Australia was shown to be non-transmissible through the seed (4). Weimer (9) reported alfalfa mosaic virus was not seed-borne in alfalfa (*Medicago sativa* L.). Hein (5) obtained no transmission of alfalfa mosaic virus through seeds of *Petunia* sp. harvested from artificially inoculated plants. From the results of experiments reported herein, there was no evidence for transmission of either alfalfa mosaic or bean yellow mosaic virus through seeds of Ladino white clover. Furthermore, seeds from virus-infected plants were not shriveled or abnormal in appearance, nor was the percentage of germination lower than that of healthy seeds.

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BROWN AND RHIZOPUS ROTS OF PEACHES AS AFFECTED BY HYDROCOOLING, FUNGICIDES, AND TEMPERATURE [1]

T. T. McClure [2]

SUMMARY

Hydrocooling with plain ice water had little or no effect on the decay of peaches inoculated with the brown rot or the Rhizopus rot fungus. Adding Clorox (sodium hypochlorite) or Dowicide A (97 per cent sodium *o*-phenylphenoxide) to the hydrocooling water significantly reduced decay. Hydrocooling peaches in a Clorox solution containing 200 ppm of chlorine reduced brown rot 29 per cent and Rhizopus rot 4 per cent. Hydrocooling with 0.1 per cent Dowicide A reduced brown rot 64 per cent and Rhizopus rot 17 per cent.

Holding non-hydrocooled peaches 5 days at 40°F had no effect on the development of brown rot during subsequent ripening at 75° but reduced Rhizopus rot 25 per cent. Decay reductions were greater when a cold holding period followed hydrocooling of peaches than when hydrocooling alone was used. A combination treatment consisting of hydrocooling peaches in 0.1 per cent Dowicide A solution followed by 5 days at 40° reduced brown rot 91 per cent and Rhizopus rot 47 per cent.

Rapid cooling of peaches by hydrocooling has become general practice in the southeastern states during the last few years. To reduce peach decay, the hydrocooling water usually contains sodium or calcium hypochlorite at a chlorine concentration of 125–200 ppm (2). Decay is not adequately controlled at these concentrations, and higher concentrations have not given statistically significant further reductions (3). A more effective fungicide is needed. Of various fungicides applied to peaches as post-harvest sprays from 1951 to 1954, Dowicide A (97 per cent sodium *o*-phenylphenoxide) controlled brown and Rhizopus rots best (8). The present laboratory study of post-harvest decay in inoculated peaches compares peaches hydrocooled in solutions containing Dowicide A or Clorox (sodium hypochlorite) with peaches hydrocooled in plain ice water. Because refrigeration during shipment is common practice, some of the test peaches were refrigerated for a short period before ripening.

MATERIALS AND METHODS.—In 1954 the peaches were obtained from a wholesale market; in 1955 and 1956 they were picked locally. Most of the tests were made with Red Haven, Early Elberta, or Elberta varieties, depending on availability.

The fruit were sorted for uniform size, ripeness, and freedom from injuries. At the start of each test, average firmness of a random sample was obtained with a Magness-Taylor pressure tester (7) with a 5/16-in. plunger.

The test fruit were inoculated just before treatment to ensure a high incidence of brown and Rhizopus rots. Uninjured peaches were dipped in a spore suspension of *Monilinia fructicola* (Wint.) Honey, the pathogen that causes brown rot. Other peaches, wounded on each cheek with a shallow knife cut, were similarly inoculated with *Rhizopus stolonifer* (Fr.) Lind.

Treatment consisted of hydrocooling peaches for 15 minutes 1) in ice water alone, 2) in ice water containing Clorox (200 ppm of chlorine), and 3) in ice water containing Dowicide A (0.1 per cent); a fourth group was held without hydrocooling. Flood-type hydrocoolers were improvised similar to commercial equipment in solution temperature and flow rate. Solution temperature was maintained between 32° and 38°F; fruit temperature after hydrocooling averaged 52.4°.

Peaches from each treatment were ripened as follows: 1) placed immediately at 75°F, or 2) held 5 days at 40°, to simulate a refrigerated transit period, before placing at 75°. A 40°F holding temperature was chosen since it is the highest temperature at which virtually no softening occurs (6).

Companion lots of uninoculated peaches were tested for firmness from time to time. When a firmness of 2 pounds or less was obtained, all similarly treated inoculated fruit were considered fully ripe (4).

The percentages of peaches decayed when fully ripe were averaged for each year and the 3-year average reductions below the decay of the checks were computed. Non-hydrocooled peaches placed immediately at 75°F were used as checks.

RESULTS AND DISCUSSION.—Hydrocooling peaches in plain ice water and ripening immediately did not significantly reduce brown rot below that in checks (Table 1). Brown rot was significantly reduced in 2 of the 3 years when peaches were hydrocooled in Clorox solution and in both years when a solution of Dowicide A was used. Results were similar when peaches were held 5 days at 40°F after hydrocooling.

When peaches were ripened immediately at 75°F Rhizopus rot was not significantly reduced by hydrocooling in plain ice water or in Clorox solution, but was significantly reduced by hydrocooling in Dowicide A solution in 1 of 2 test years.

A cold holding period between treating and ripening non-hydrocooled peaches did not reduce brown rot but

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TABLE 1.—*Brown and Rhizopus rots of inoculated peaches ripened after various hydrocooling and fungicidal treatments*

Treatment after inoculation	Brown Rot: Average per cent of inoculated peaches infected			Average per cent reduction of infection below that in check	Rhizopus Rot: Average per cent of inoculated peaches infected			Average per cent reduction of infection below that in check
	1954	1955	1956		1954	1955	1956	
Immediate ripening at 75°F:								
No hydrocooling (check)	90	48	93	—	90	99	97	—
Hydrocooling in ice water								
Without fungicide	95	50	82	1	93	99	98	—1[a]
With Clorox[b]	53**	42	63**	29	85	94	97	4
With Dowicide A[c]	—	21**	26**	64	—	82	81*	17
Ripening at 75° after 5 days at 40°:								
No hydrocooling	92	46	89	2	68	89	59**	25
Hydrocooling in ice water								
Without fungicide	92	49	64**	9	59**	95	90	15
With Clorox[b]	61**	25*	40**	46	56**	80*	73**	27
With Dowicide A[c]	—	5**	8**	91	—	56**	49**	47

* and ** indicate statistically significant differences from the checks at the 5 per cent and the 1 per cent levels, respectively.

[a] Minus sign indicates a negative reduction (increase).

[b] Clorox at 200 ppm of chlorine.

[c] Dowicide A at 0.1 per cent concentration.

reduced Rhizopus rot 25 per cent. Results were similar when peaches were hydrocooled in ice water without fungicide. The effectiveness of both fungicides was enhanced by a cold holding period after treatment.

Since no Rhizopus rot and very little brown rot can develop during 5 days at 40° (1), the only time available for decay to develop was the ripening time at 75°. It was of interest to determine whether decay differences could be correlated with ripening time. Hydrocooling in plain ice water had practically no effect on softening of peaches during a subsequent 2-day period at 75° (Table 2) or on reducing either decay (Table 1). On the other hand, peaches subjected to a cold holding period are known to ripen more rapidly on removal than those not refrigerated (5, 6). This effect was observed in the present experiments. If the cold holding period reduced decay by shortening the ripening time, leaving less time for decay to develop, then the slower-growing *Monilinia* should have been affected more than the faster-growing *Rhizopus*. But the opposite occurred. Decay reduction from a cold holding period is apparently not due primarily to a shortening of the time available for decay development.

TABLE 2.—*Average firmness of non-hydrocooled and hydrocooled peaches held at 40°, 75°F, or both*

Original firmness and holding temperature (°F)	Holding period (days)	Firmness after holding: Non-hydrocooled (lb.)	Firmness after holding: Hydrocooled (lb.)	Differences due to hydrocooling (lb.)
14.2 pounds				
40	5	13.2	14.0	0.8
75	2	4.8	5.3	0.5
40 + 75	5 + 2	2.0	2.1	0.1
6.8 pounds				
40	5	3.5	4.6	1.1
75	2	1.1	1.2	0.1
40 + 75	5 + 2	0.8	0.9	0.1

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INFLUENCE OF CALCIUM AND BORON NUTRITION ON DEVELOPMENT OF FUSARIUM WILT OF TOMATO [1]

L. V. Edgington [2] and J. C. Walker

SUMMARY

Bonny Best tomato plants inoculated with *Fusarium oxysporum* f. *lycopersici* showed a progressive decline in severity of wilt symptoms with increase of calcium from 5 to 500 ppm. Boron levels in a range of 0.001–10 ppm affected the trend, but not progressively: with calcium at 5 ppm, the disease was very severe at both 0.001 and 10 ppm boron; with calcium at 100 ppm, the disease index decreased significantly from 0.001 to 0.25 ppm boron; with calcium at 500 ppm, the index increased consistently, and usually significantly, with increase in boron from 0.001 to 0.25 and to 10 ppm.

Nutritional studies on *Fusarium* wilt of tomato (*Lycopersicum esculentum* Mill.), incited by *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyder and Hansen, have been conducted by numerous workers to find a nutritional amendment and gain a better understanding of the basic physiology of the disease. Fisher (3) reported that signs of the disease failed to appear if boron was omitted from the nutrient supplied to tomato plants, beginning 1 week after inoculation with the fungus. An excessive amount of calcium in the nutrient markedly reduced the amount of vascular browning. The present research was initiated to study the effects of different levels of calcium on disease development. The effects of different levels of both calcium and boron were then investigated, since Reeve and Shive (6) and Brennan and Shive (1) have shown that an interrelation occurs between the intake of boron and calcium by roots of tomato. Thus, as the concentration of calcium in the substrate increased, the relative intake of boron decreased, as shown by tissue analysis. This was particularly striking in all aerial parts of the plant at a high level of boron (5 ppm) in the substrate, and in the apical leaves at a low level (0.001 ppm) of boron.

MATERIALS AND METHODS.—Seed of Bonny Best tomato was sown in vermiculite. Three-week-old plants were transplanted to white quartz sand in a continuous-drip system, as described by Gallegly and Walker (4). Plants were supplied with Hoagland's solution or modifications thereof. When the calcium concentration, supplied as calcium nitrate in Hoagland's solution, was decreased, the nitrate was kept constant by adding sodium nitrate. Calcium chloride was added, to give high levels of calcium. The osmotic value of the various solutions was kept constant by addition of sodium chloride. When boron concentration was altered, no other nutrients were modified. Inoculum was prepared by growing the fungus in modified Richard's solution for 2 weeks in shake culture. The culture was washed by 2 centrifugations and resuspended in tap water. Plants were inoculated 15–18 days after transplanting, by lifting them from the sand, washing and dipping their roots in the fungal inoculum, and replanting them in the same pots.

Disease ratings were made 17–20 days after inoculation. The plants were placed in one of the following disease classes: 0, plants healthy; 20, 1 leaf showing wilt of some or all leaflets; 40, 2 to several leaves wilted on lower half of plant; 60, all leaves wilted except growing tip and leaves immediately below; 80, all leaves wilted; 100, plant dead. A disease index for each treatment replicate was obtained by multiplying each disease class number by the number of plants in the class and dividing the sum of the products by the total number of plants.

EXPERIMENTAL RESULTS.—*Influence of calcium on disease incidence.*—Three experiments were conducted in which calcium was supplied at concentrations of 13, 20, 40, and 160 ppm during both pre- and post-inoculation periods. Results are presented in Table 1. Plants receiving 13 or 20 ppm were noticeably stunted at the time of inoculation and, in 2 of the 3 trials, were significantly more susceptible to wilt than were plants receiving 160 ppm. Except for some mild marginal chlorosis of leaves, plants receiving 40 ppm developed no marked symptoms of deficiency. Although differences were not significant, these plants consistently had higher disease indices than those with 160 ppm of calcium.

In general, smaller plants appeared to be more susceptible to *Fusarium*. Because the plants receiving low levels of calcium were smaller than those receiving 160 ppm at the time of inoculation, 2 experiments were conducted in which plants received a complete nutrient solution until inoculation. After inoculation, calcium was completely deleted in 1 treatment while plants in the other treatment continued to receive the normal amount. Although differences in disease indices were not significant, post-inoculation calcium-deficient plants were consistently more susceptible than plants receiving normal calcium.

Influence of variation of calcium and boron in the nutrient.—The "normal" balanced Hoagland's nutrient solution used in this series contained 0.25 ppm of boron and 100 ppm of calcium. To test the effect of

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TABLE 1.—*Development of Fusarium wilt in tomato plants supplied with Hoagland's nutrient solution adjusted to various levels of calcium*

Calcium (ppm)	Disease index[a] in five experiments: Calcium supplied before and after inoculation 1	2	3	Calcium supplied after inoculation 4	5
0				73	62
13	48	46	48		
20	45	48	38		
40	37	38	18		
160	26	32	12	50	44
LSD 19:1	14	NS[b]	12	NS	NS
99:1	25	NS	23	NS	NS

[a] Based on a scale of 0 to 100 in which 0 indicates no disease and 100 indicates all plants dead. Each index figure is the average of 2 replicates of 40 plants each.
[b] Differences not significant.

TABLE 2.—*Development of Fusarium wilt in tomato plants supplied with various concentrations of boron and calcium in Hoagland's nutrient solution*

No.	Nutrient treatment: Boron (ppm)	Calcium (ppm)	Disease index[a]: Exp. 1	Exp. 2
1	0.001	5	100	94
2	0.001	100	72	60
3	0.001	500	33	14
4	0.25	100	56	40
5	0.25	500		28
6	10.0	5	100	99
7	10.0	500	68	70
		LSD 19:1	12	18
		99:1	18	26

[a] Based on a scale of 0 to 100 in which 0 indicates no disease and 100 indicates all plants dead. Each figure represents the average index of 2 replicates of 20 plants each.

various combinations of the 2 elements, a low level (0.001 ppm) and a high level (10 ppm) of boron were used. The low calcium level selected was 5 ppm, and the high level was 500 ppm. Plants were supplied with normal solution until inoculation and treatments were begun, immediately after inoculation. The 7 treatments are listed in Table 2, in which the "normal" solution is that used in treatment 4. Two experiments were run, each containing 2 replicates of 20 plants each. In the plants at 0.001 ppm of boron, deficiency symptoms developed in the form of apical necrosis and leaf malformation at all 3 levels of calcium. The boron content of apical portions, as determined by the method of Dible, Truog, and Berger (2), was about the same in boron-deficient plants at all levels of calcium; in plants supplied with normal solution, the boron content was about 3 times as high. The plants supplied with low boron and low calcium showed a pronounced chlorosis, accompanied by a purpling of the veins in some leaflets. In low-boron nutrient, these symptoms decreased in intensity with increase in calcium level. This trend could not be ascribed to less intake of boron at the high calcium level, as reported by Brennan and Shive (1), since the analysis showed approximately the same amount of boron in plants at the 3 levels of calcium in the substrate. The chlorosis and purpling of veins developed more rapidly in inoculated than in uninoculated plants, but did not occur in plants growing in normal solution. Boron toxicity symptoms in the form of marginal necrosis of leaflets appeared in plants supplied 10 ppm of boron, in combination with either 5 or 500 ppm of calcium.

The disease indices are presented in Table 2. At each respective boron level the disease index decreased with each increase in calcium. This is in accord with the result of Fisher (3), who secured a reduction in vascular browning when an excess of calcium was supplied.

When the effect of different levels of boron at respective levels of calcium is considered, there was not a progressive trend. The disease index was very high at low and high boron levels when calcium was low (5 ppm). When the calcium was normal (100 ppm) the index decreased significantly from 0.001 to 0.25 ppm of boron. However, when calcium was in excess (500 ppm), the index increased progressively from 0.001 to 10 ppm. of boron. Insofar as the results can be compared with those of Fisher (3), they contradict them: when he omitted boron from an otherwise normal solution, no symptoms were observed, whereas reduction of boron in this study from 0.25 ppm to 0.001 ppm increased the index significantly.

DISCUSSION.—It may be concluded that an increase in calcium in the nutrient definitely retards the development of wilt. This trend was the same at all 3 levels of boron in the nutrient. It is of interest to note that Reeve and Shive (6) concluded that the calcium content of tomato tissue was determined by the calcium content of the substrate and appeared independent of boron in the substrate. Although calcium concentration in the substrate did not affect boron uptake quantitatively, as obtained by Brennan and Shive (1), the influence of boron on disease was dependent upon the calcium supply. Calcium is an important constituent of middle lamellae, and it has been shown that boron affects the thickness of cell walls in certain tissues of celery (7). It would be of interest, therefore, to investigate further whether variation of these 2 elements in the substrate and in the tissues has an effect on the production of fungus metabolites that induce various phases of the wilt disease and/or on the relation of such metabolites to wall constituents and the cell permeability of the host.

In this connection it is worthy of note that Holden (5) found the activity of fungal polygalacturonase on tobacco leaf fiber substrate to be enhanced by pretreating the fiber with 0.5 M ammonium acetate, sodium acetate, or potassium acetate. This was attributed to the replacement of calcium in pectin by one of the monovalent ions. In the present instance, an increase of calcium in the substrate might work in the opposite manner, enhancing the binding of pectin

chains and thereby reducing polygalacturonase activity, with consequent retardation of wilt development.

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ETIOLOGY AND EPIPHYTOLOGY OF NORTHERN ANTHRACNOSE OF RED CLOVER [1, 2]

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SUMMARY

Infection occurred when red clover plants of the Pennscott variety were exposed to a moisture-saturated atmosphere for as little as 3 hours after inoculation with conidia of *Kabatiella caulivora* (Kirch.) Karak. The per cent of infection increased with time of exposure up to 60 hours, the longest period tested. No infection was obtained when plants were sprayed with aqueous conidial suspensions containing less than 125,000 spores per ml.

On the host, spore germination began 15 hours after inoculation. Appressoria appeared in 48 hours, and penetration of epidermal tissues occurred 48–96 hours after inoculation. Progress of the fungus within the host was both intercellular and intracellular. At 20°C, macroscopic symptoms appeared 8 days after inoculation.

All above-ground plant parts were infected, including leaves, stipules, petioles, main stems, floral heads, and flowers. Floral-head infection resulted in the production of immature, shriveled seed. Seed transmission of the organism was demonstrated when seeds from a severely infected stand of red clover were planted and anthracnose-diseased seedlings were obtained. Wind dissemination of conidia could not be demonstrated. The survival potential of *K. caulivora* was demonstrated by the longevity of conidia on seed in storage for periods up to 240 days and in soil for periods up to 300 days. Conidia survived for 380 days on glass coverslips at 3°, 6°, and 9°, and for 20 months at −10°C. The fungus was recovered from diseased plant debris stored outdoors for 400 days.

Forty-three species, representing 4 genera in the *Leguminosae*, were tested as possible hosts. Infection was obtained following inoculation on *Trifolium pratense* L., *T. repens* f. *giganteum* Lagr.-Foss, *T. dubium* Sibth., *T. incarnatum* L., *T. hirtum* All., *T. alexandrinum* L., *T. glomeratum* L., *T. angustifolium* L., *T. medium* Huds., *T. pallidium* L., *T. fragiferum* L., *Melilotus alba* Desr., and *Medicago sativa* L.

Northern anthracnose, incited by *Kabatiella caulivora* (Kirch.) Karak., is one of the major diseases of red clover (*Trifolium pratense* L.) in the cool, moist regions of North America, Europe, and Asia. During climatic periods favorable for development of the disease, hay quality and quantity are both reduced. Reduction in yield is greatest in the second and later cuttings. Severely infected plants produce few seeds or none, making seed production generally unprofitable in areas where anthracnose is found.

The following investigation was undertaken to clarify understanding of the year-to-year survival of the northern anthracnose pathogen, the spread of the pathogen in nature, the process of host colonization, and the production of disease reactions. All experiments were performed with the conidial, yeast-like-growth form of *K. caulivora* (9).

Means by which the pathogen survives its adverse season.—*Survival as free conidia on seed.*—Sampson (9) reported conidia of *K. caulivora* survived 18 months on artificially contaminated red clover seed stored at room temperature, and that diseased seedlings developed when this infested seed was planted. Minyaeva (7) found that, after 9 months storage, only 0.28 per cent of the artificially contaminated seed yielded viable conidia as compared with 92.5 per cent at the beginning of the storage period. Seedlings from

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contaminated clover seed sown in soil were 16.9 per cent diseased.

In 1902, Bolley (2) developed a technique for determining the presence, on seed of their respective hosts, of spores of the wheat bunt organism, *Tilletia foetida* (Wallr.) Liro, and of the flax wilt fungus, *Fusarium oxysporum* f. *lini* (Bolley) Snyd. & Hans. The seed was washed with distilled water, the wash water was centrifuged to accelerate sedimentation, and the sediment was examined through a microscope for the spores in question. Recently, an adaptation of this technique was successfully used by Couch and Grogan (3) in studies of the lettuce anthracnose organism, *Marssonina panattoniana* (Berl.) Magn.

In our investigation 500 g of anthracnose-free red clover seed of the Kenland variety was sprayed with a conidial suspension of *K. caulivora*, allowed to air dry, and stored at room temperature (23–30°C). After each of 10 storage periods, varying from 10 to 270 days (Table 1), 30-g seed samples were washed with 100 ml of sterile distilled water and the wash water centrifuged 10 minutes at 5000 rpm. The sedimented conidia were then re-suspended in 10 ml of water. This procedure provided concentrated suspensions containing about 1,000,000 conidia per ml. The suspensions obtained were sprayed on 100 5-week-old red clover plants of the Pennscott variety. The inoculated plants, after 24 hours in a moisture-saturated atmosphere at 17°C, were maintained in a greenhouse at 20–23°C. Fifteen days after inoculation, readings were taken as per cent of plants infected, the criterion for infection being the presence of at least 1 lesion per plant. In addition, after each of the 10 storage periods mentioned above, samples of about 500 infested seed were planted in flats in the greenhouse at 20°C. After germination, the seedlings were removed to a moist chamber at 17°C for 4 days to promote infection, and then returned to the greenhouse. Conidia washed from infested seed infected healthy plants after storage periods up to 240 days, but not after 270 days. When sown, infested seed that had been stored for periods up to 210 days produced diseased seedlings (Table 1). Symptoms were readily apparent on the seedlings 9 days after germination. The parts attacked included cotyledons, the unifoliate leaf and petiole, and the first trifoliate leaves.

TABLE 1.—*Longevity at 23–30°C of conidia sprayed on red clover seed as evidenced by bioassay*

Storage period (days)	% diseased seedlings from infested sample[a]	% plants infected by spore washings
10	12	33
30	13	30
60	9	23
90	11	24
120	7	20
150	4	18
180	5	13
210	1	5
240	0	1
270	0	0

[a] 500 seeds per sample.

Survival in debris of previously infected plants.—Wellensiek (12) reported that *K. caulivora* survived at least 2 weeks in leaf stalks buried in sterile soil at −22°C. Sampson (9) stated that Jenkins found infected petioles bearing acervuli in the field as late as December. In the present study, infected plants were collected from a field of Pennscott red clover on September 29, 1954. Part were stored at room temperature (23–30°C); the rest were placed outdoors in an exposed location. To isolate the organism, tissue sections containing lesions were surface-sterilized with an aqueous 0.10 per cent mercuric chloride solution, placed in potato-dextrose-yeast agar, and incubated at 20°C. The fungus was recovered from plant debris held at room temperature for 320 days, but not for 350 days, and from material stored outdoors for 400 days, but not for 450 days.

Survival of free conidia in soil.—Wellensiek (12) reported that clover plants became severely diseased if grown in soil that had been previously contaminated by spraying with a conidial suspension.

In the present investigation, samples of sterilized (autoclaved 1 hour at 120°C) and unsterilized Hagerstown silt loam (moisture equivalent 24.5 per cent) were infested with concentrated spore suspensions of *K. caulivora*, after which sufficient water was added to bring the soil to its moisture equivalent. From the soil samples, stored in air-tight containers at 18–20°C, portions were removed at monthly intervals, wetted to a liquid consistency, and brushed on the petioles and leaves of healthy Pennscott plants. The plants, after 24 hours in a moist chamber at 20°C, were maintained in a greenhouse at 20–23°C. Disease readings were taken 15 days after inoculation. Infection was obtained from both sterilized and unsterilized soil stored for periods up to 300 days. After longer periods, neither soil sample produced infection.

Survival of conidia on glass coverslips.—Conidial survival was investigated at 3°, 6°, 9°, 12°, 15°, 18°, and 24°C. Samples of conidia were transferred to sterile glass coverslips with a transfer needle. The coverslips were then placed in Petri dishes within desiccator jars. At each temperature, spores were stored at 0 vapor pressure deficit (1), and at 5 mm Hg vapor pressure deficit (VPD). Humidity control was obtained by placing in the bottom compartment of the desiccators, sulfuric acid solutions of the specific gravity necessary to maintain the desired vapor pressure deficit at each temperature. Data for selection of the proper acid concentrations were obtained from the International Critical Tables Volume 1 (11). Conidia also were maintained in the frozen state at −10°C. To determine survival, coverslips were removed periodically from each temperature and humidity condition and inserted into potato-dextrose-yeast agar. Formation of a visible colony of *K. caulivora* was the criterion for spore viability.

Conidia survived 380 days at 3°, 6°, and 9°C at both

FIG. 1. Natural infection of floral head of Pennscott red clover with *K. caulivora*. Left, diseased; right, healthy.

0 VPD and 5mm Hg VPD. At 12°, 15°, and 18°C the conidia survived 360 days at 5 mm Hg VPD, but survival at 0 VPD could not be demonstrated for a period longer than 150 days. At 24°C, conidia survived 150 days at both vapor pressure deficits. Conidia in the frozen state were still viable after 20 months at −10°C.

INFECTION STUDIES.—*Length of exposure to saturated atmosphere required for plants to become infected.*—Northern anthracnose epiphytotics are associated with wet weather (9, 12). Three experiments were conducted to determine how long plants must be exposed to a moisture-saturated atmosphere for infection to be accomplished. Following the method of Couch and Grogan (3), healthy red clover plants of the Pennscott variety were sprayed with an aqueous suspension (2,000,000 conidia per ml) of *K. caulivora* and placed in a moisture-saturated atmosphere at 20°C. At each of 12 intervals, from 1 to 60 hours, 5 pots containing 20 plants each were removed and dried with an electric fan, after which they were stored at 20°–23°C.

In 2 experiments, infection occurred after a 5-hour exposure to a moisture-saturated atmosphere; in the third, infection occurred after a 3-hour exposure. The per cent of plants infected increased with increased exposure up to the longest period tested, 60 hours. With this period of exposure, 33 per cent of the plants became infected.

Inoculum density.—Two experiments were conducted to determine how inoculum density influenced infection. Six-week-old plants of the Pennscott variety were sprayed with aqueous conidial suspensions of various concentrations. The most concentrated suspension contained 4,000,000 conidia per ml. This was diluted serially to provide 11 aliquots, the lowest concentration being 2,000 conidia per ml. Before each suspension was used, its final spore density was checked with a Levy counting chamber. The foliage and petioles of 100 plants were sprayed to run-off point with each concentration, held 24 hours at 20°C in a moist chamber, and then removed to a greenhouse at 20–23°C. Disease ratings were made 21 days after inoculation. The development of at least 1 lesion per plant was used as the criterion for successful infection.

No infection was obtained in either experiment with spore concentrations of 63,000 per ml or lower. At the 125,000-per-ml level, 3 per cent of the plants became infected in the first experiment and 4 per cent in the second. At the maximum spore concentration of 4,000,000 per ml, 30 per cent and 48 per cent infection were obtained, respectively.

Infection and disease development.—Concerning infection, Sampson (9) reported that, "On the host the spore sends out a slender germ tube which grows at first in the cell wall immediately below the cuticle. Growth in the host is preceded by brown discoloration of the cell walls and eventually the cells have brown contents. The mycelium is almost entirely intercellular."

In the present study, petioles and leaves of six-week-old plants of the Pennscott variety were inoculated with an aqueous conidial suspension and placed in a moist chamber at 20°C. At 4, 12, 18, 24, 48, 72, 96, 120, and 144 hours after inoculation, leaf sections and petiole strippings were taken. These tissues were cleared by immersion for 20 minutes, at a temperature just below boiling, in a lacto-phenol solution containing 0.10 per cent cotton blue (3).

Microscopic examination of the tissues showed that spore germination, as evidenced by germ-tube forma-

tion, began 15 hours after inoculation. Appressoria were observed 48 hours after inoculation, but actual penetration of epidermal tissues did not occur until 48–96 hours after inoculation. Penetration was direct; entry through stomates was never observed. Cuticular penetration appeared to be mechanical. The infection hyphae were never seen to enter the lumina of epidermal cells, but remained intercellular. The contents of adjacent epidermal cells became brown and discolored. After 72 hours, little mycelial proliferation was observed. The most striking evidence of the fungus' advance was the browning and ultimate collapse of cells. Percentage of spore germination on the host was extremely low, always less than 1 per cent.

Progress of the fungus within the host was studied by means of serial sections. Inoculated petioles showing typical lesions were fixed and killed in formalin-aceto-alcohol (6), embedded in paraffin, and sectioned on a rotary microtome. The sections were stained with 1.0 per cent aqueous phloxine for 12 hours, dehydrated, counterstained with 0.3 per cent fast green in absolute ethyl alcohol for 1 minute, and cleared with clove oil. Microscopic study of the sections showed the fungus to be both intercellular and intracellular, and not restricted to specific tissues. The vascular bundles remained relatively intact although the collapse of surrounding parenchymatous cells caused them to be displaced.

The first macroscopic symptoms of the disease appeared 8 days after inoculation. Petiole and stem infection was characterized by the formation of a slightly depressed dark-brown streak, which gradually enlarged to form an elongated dark-brown to black lesion. Greyish-white masses of conidia and conidiophores became conspicuous within the central depressed area of the lesions after 10–20 days. In many instances, the weakened petiole bent at the lesion site, producing the typical "shepherd's crook" described by Sampson (9). With foliar infection, the leaflet veins darkened and became prominent; brown, dry areas appeared on the lamina, followed by considerable curling. In some instances, infection and shriveling of the petiolules at the point of attachment caused the leaflets to wilt, producing a drooped appearance. Microscopic examination of lacto-phenol-cleared leaflets showed conidiophores and fungal hyphae growing from stomates. In this manner, secondary inoculum is actually produced prior to the formation of characteristic acervular lesions containing conidial masses.

Floral infection and seed transmission.—Sampson (9) stated that infection of the calyx and corolla of red clover was never observed. However, Sigrianski and Potapova (10) reported that brown lesions were produced 10–15 days after inoculating the flowers. In 1951 Minyaeva (7) found that seed from infected plants bore dark discolored spots. Colonies of *K. caulivora* developed from 1.89 per cent of the spotted seed when sown on sterile nutrient media. Wellensiek (12), however, reported that he was unable to find northern anthracnose in over 3,000 plants grown from seed harvested from 32 diseased fields. Sampson (9) found no evidence of seed transmission from 52 seed samples from red clover fields of 12 countries.

Positive evidence of flower infection and transmission of the pathogen through uncleaned seed was found in the present investigation. On red clover in Pennsylvania, during the 1956 growing season, lesions were observed on all above-ground plant parts, including leaves, stipules, petioles, main stems, and floral heads. The disease on the floral heads included infection of the individual flowers (Fig. 1). *K. caulivora* was successfully isolated from portions of these floral heads. Infected floral heads generally produced shriveled, brownish seed or plump seed with dark brown spots. If the supporting petiole was attacked early in floral ontogeny no seed were produced. If infection occurred later, but before maturity, shriveled seeds were produced.

In September of 1956, a combine-harvested seed sample was collected from a severely diseased 2-year-old red clover planting on the University Farms. Northern anthracnose had been present in the first cutting (prior to the July harvest) and, because of cool, moist weather, increased up to the September seed harvest. The seed samples contained considerable foreign matter, i.e., stem pieces, shattered leaves, petioles, and dried floral parts. About 75 per cent of the seed was shriveled or discolored.

Portions of the combine-harvested sample, containing debris and shriveled and sound seed, were planted in flats of sterile soil. After the seeds germinated, the flats were removed to a saturated atmosphere at 20°C for 4 days and then returned to a greenhouse bench. Of the total of 4,000 plants, 15 diseased seedlings were observed. Infection was typified by a darkening of the leaf bases and a shriveling of the petioles of the the primary and trifoliate leaves, the same as symptoms observed on seedlings grown from artificially infested seed. *K. caulivora* was isolated from the diseased seedlings.

Spread of fungus in nature.—Wind dissemination of spores in the order *Melanconiales* is uncommon, for they are generally produced in sticky, acervular masses. Wilson (13) demonstrated that conidia of *Coryneum beijerinckii* Oud. are rarely detached by dry air, but readily by water drops. Couch and Grogan (3) have shown that conidia of *Marssonina panattoniana* may be dislodged from wet acervuli and carried in a wind stream. With *K. caulivora* it has been shown that conidia are easily dislodged by water or falling water droplets (9).

The following experiment was conducted to determine if conidia of *K. caulivora* might become windborne. Stem sections with mature lesions were used as the source of spores. An electric fan provided an air current that passed over the lesions and then over 10 pots of healthy red clover, 2 feet away. The leaves of the test plants were kept moist throughout exposure by air current. In 1 trial, the lesions were dry; in another, they were kept moist. After treatment, the

exposed plants were removed to a moist chamber at 20°C for 24 hours and then returned to a greenhouse at 20–23°C. The plants were checked at intervals for 25 days following exposure. No evidence of infection was observed.

Influence of air temperature on infection.—Monteith (8) reported that 20°C is optimum temperature both for growth of *K. caulivora* in culture and for infection of red clover. In the present investigation, red clover plants of the Pennscott variety were inoculated by spraying foliage and petioles to run-off point with an aqueous suspension of 1,000,000 conidia per ml and then held at different temperatures. One series of 100 plants was held in a moist chamber at 19–21°C for 24 hours and then removed to a greenhouse at 20–23°C. A second series of 100 plants was held in a moist chamber at 15–17°C for 24 hours and then removed to a room at the same temperature. A third series of 100 plants was maintained at 25–28°C following inoculation and throughout incubation.

Final disease ratings were made 20 days after inoculation, with 1 lesion being considered sufficient to rate a plant as being infected. Thirty-two of the plants held at 20–23°C became infected; symptoms appeared 8 days after inoculation. Nine plants of the 15–17°C series became diseased; symptoms appeared within 12 days. Only 1 plant of the 25–28°C series developed anthracnose symptoms. These results indicate that relatively low temperatures and long periods of high humidity are most favorable for disease production.

Host range of the pathogen.—Regarding the host range of *K. caulivora*, Fulton (4) obtained successful infection on *Trifolium hybridum* L. and *T. pratense* L. but was unable to produce the disease on *T. repens* L. and *Medicago sativa* L. Infection experiments were also performed by Sampson (9), who reported typical anthracnose lesions were produced following inoculation on *Trifolium pratense* L., *T. hybridum* L., *T. repens* L., and *T. suaveolens* L. She obtained negative results, however, with *T. medium* Huds., *T. incarnatum* L., *Medicago sativa* L., *M. lupulina* L., *Lathyrus odoratus* L., *Vicia faba* L., *Glycine max* (L.) Merr., *Phaseolus multiflora* L., and *P. vulgaris* L. Hanson (5) reported that *T. pratense* L., *T. alexandrinum* L., and *T. lappaceum* L. became severely infected, while moderate infection developed on *T. fragiferum* L., *T. hybridum* L., *T. incarnatum* L., *T. medium* Huds., *T. repens* L., *T. resupinatum* L., *T. striatum* L., *T. subterraneum* L., and *T. wormskioldii* Lehm.

In this study, 44 species, representing the genera *Trifolium* L., *Melilotus* Mill., *Medicago* L., and *Lotus* L., were investigated as possible hosts for *K. caulivora*. One hundred 30-day-old plants of each species, inoculated by spraying with an aqueous conidial suspension of *K. caulivora*, were kept in a moisture-saturated atmosphere for 48 hours at 20°C, then removed to a greenhouse at 19–21°C. Typical lesions developed on *Trifolium dubium* Sibth., *T. pratense* L., *T. incarnatum* L., *T. repens* L., *T. hirtum* All., *T. alexandrinum* L., *T. glomeratum* L., *T. angustifolium* L., *T. medium* Huds., *T. pallidium* L., *T. fragiferum* L., *Melilotus alba* Desr., and *Medicago sativa* L. In all cases of disease development, the fungus was re-isolated from host lesions. No lesions occurred following inoculation of *Lotus corniculatus* L., *Medicago falcata* L., *M. lupulina* L., *M. minima* Gluff., *M. scutellata* All., *Melilotus indica* All., *M. officinalis* (L.) Lam., *Trifolium agrarium* L., *T. amabile* H.B.K., *T. ambiguum* M. Bieb, *T. arvense* L., *T. carolinianum* Michx., *T. corniculatus* L., *T. hybridum* L., *T. ismocarpum* Drot., *T. lapaceum* L., *T. nigrescens* Viv., *T. pamonicum* L., *T. phleoides* Huds., *T. reflexum* L., *T. repens* L. (Louisiana white), *T. resupinatum* L., *T. lupellianum* Viv., *T. scutatum* Br., *T. semipillosum* Eat., *T. striatum* L., *T. subteranneum* L., *T. subratundum* L., *T. tembense* L., *T. variegatum* Nutt., and *T. wormskjoldii* Lehm.

Discussion.—The primary limiting factors for epiphytotic development of northern anthracnose appear to be temperature and moisture, with continuous periods of cool, wet weather being most favorable for disease development. Maximum growth in culture and highest per cent of infection are produced in the narrow range of 18–22°C. Above the optimum temperatures, infection is almost completely inhibited; below, symptom development is slowed and fewer plants become infected. Although infection occurs when inoculated plants are exposed to a saturated atmosphere for as short a period as 3 hours, greatest infection results from 24–60 hours of exposure.

Inoculum density greatly influences the amount of infection obtained. The variation in infection percentage from variation in inoculum concentration is fully as great as that which could be expected from race and host strain variation. In view of this, screening for resistance and race determinations should be performed with conidial suspensions of uniform concentration.

K. caulivora may remain viable for long periods, as shown by its longevity in debris and soil, on seed, and on glass coverslips. The form in which the organism survives in debris is not established. Conidia are the only spore form that has been observed, but the possibility also exists that mycelium remains viable for long periods. Conidia are surrounded by a thick transparent coating that evidently aids them in withstanding adverse conditions.

Spread of the pathogen in nature takes place over short distances, by rain and splashing water. Since conidia of the pathogen are apparently not wind-disseminated, long-distance spread into new areas probably occurs through the use of seed from infected plantings and the movement of infested red clover hay.

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THERMAL STABILITY OF PEACH ROSETTE VIRUS [1]

Glenn KenKnight [2]

SUMMARY

Stems or entire trees of peach and plum inoculated with peach rosette virus (PRV) were given hot-water-bath treatments. PRV transmission did not result with bark patches from stems treated longer than 18 minutes at 50°C. In an experiment employing entire trees, the thermal death times of PRV appeared to be less than 90 minutes at 45°, about 18 minutes at 50°, and about 2 minutes at 55°C. However, grafting nurse trees to rosetted or rosette-inoculated trees showed the thermal death time of PRV to be over 60 minutes at 50° and 10 minutes at 56°C.

Thermal death times have been determined for several stone fruit viruses. The method usually employed consisted of submerging bud sticks of infected trees in hot-water baths, but Hutchins used entire 2-year-old trees (3). Kunkel (4) reported that the thermal death time of peach rosette virus (PRV) in bud sticks is about 10 minutes at 50°C. At Fort Valley, Ga., variable results from heat treatments of PRV led to development of a new technique, the "nurse-tree" method described in this paper, whereby it was demonstrated that the thermal death time of PRV exceeds that of its host.

Methods.—Stem sections, scions, or whole trees were submerged in water in a tank equipped with electrical immersion heaters, operated by mercury thermostats accurate to 0.1°C, and with electrically operated stirrers. The thermometers were corrected to a National Bureau of Standards calibrated thermometer. The experimental trees were maintained in a screen house or in field plots at the U. S. Horticultural Field Laboratory, Fort Valley. Many uninoculated trees in the screen house and in the field plots served as control trees. None of the controls became infected. The peach trees included Lovell seedlings and several budded varieties, all of which appeared to be equally susceptible to PRV by graft inoculation.

Results.—*Experiment 1, rosetted plum scions.*—In February, 1949, scions of naturally infected *Prunus angustifolia* Marsh. trees, treated at 50°C for several periods, were grafted on peach seedling test trees. All the scions died, but transmission was obtained from a few, including 1 of 5 scions treated 12 minutes, the longest period included in the experiment.

Experiment 2, rosetted peach stems.—In October, 1953, branches from rosetted peach trees were treated at 50°C for 1, 2, 4, 8, 10, or 12 minutes. Bark patches from these branches were grafted on 2-year-old peach seedlings—4 for each treatment. In the respective groups, 3, 4, 4, 1, 4, and 1 trees developed rosette symptoms.

Experiment 3, rosetted peach trees.—In November, 1953, entire small rosetted peach seedlings were treated at 50°C for 6, 8, 10, 16, 20, or 30 minutes. All died.

Experiment 4, 2-year-old rosetted peach trees.—1) *First part.*—In the last week of December, 1953, entire 2-year-old Hiley peach trees were treated at 40° for 12–30 hours and at 45°, 50°, and 55°C for relative periods of time assuming for the virus a logarithmic thermal death line. Inoculated the preceding April with PRV by bark-patch grafts, the trees had expressed symptoms before leaf fall. Although most of the trees were dead or dying by April 30, 1954, a few survived and thereafter produced normal growth. In the 40° series, all trees died without appreciable growth. In the other series the apparent thermal

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TABLE 1.—*Effect of heat treatments on further expression of symptoms in partially to fully rosetted 2-year-old Hiley peach trees*

Treatment	Number of trees treated	Produced rosetted growth, died	Produced normal growth, died	Too little growth for diagnosis, died	Normal growth, lived
40°C					
12 hr.	5	0	0	5	0
15	5	0	0	5	0
18	5	0	0	5	0
24	5	0	0	5	0
30	5	0	0	5	0
45°C					
1½	5	0	0	0	5
2	5	0	1	4	0
3	5	0	1	3	1
4	5	0	1	4	0
5	5	0	0	5	0
50°C					
4 min.	2	1	0	1	0
6	2	2	0	0	0
8	5	4	0	1	0
10	8	3	2	3	0
12	7	1	2	3	1
15	13	3	3	7	0
18	6	0	1	5	0
20	4	0	0	4	0
30	2	0	0	2	0
60	2	0	0	2	0
55°C					
100 sec.	5	3	1	1	0
120	5	0	0	5	0
150	5	0	0	4	1
180	5	0	0	2	3
200	5	0	0	4	1
Control (untreated)	22	8	0	14	0

death time of PRV was less than 90 minutes at 45°, about 18 minutes at 50°, and 120–150 seconds at 55° (Table 1).

2) *Second part.*—In April, 1954, the apparently dead or dying trees in the 50° series were lifted. All except 1 had live roots relatively free from necrosis. Root bark from these trees was grafted on peach index trees—4 index trees per period except for the 60-minute period, which was omitted. In the respective groups treated for 4, 6, 8, 10, 12, 15, 18, 20, or 30 minutes, 3, 3, 1, 3, 0, 1, 0, and 0 index trees developed rosette symptoms.

Experiment 5, stems of incubation-stage peach trees.—In April, 1954, peach seedlings averaging 27 inches tall were pruned to whips. Twenty were inoculated with a bark patch at the center of the stem. The remaining seedlings in the row were employed as index trees or controls. One week later, half of the inoculated trees were decapitated near the soil line. Bark patches taken from the pruned stems were grafted on 136 index trees. Neither the suckers that grew from the 10 stumps nor any of the index trees developed rosette through 1956.

The remaining 10 inoculated trees were decapitated 3 weeks after inoculation. Sucker growth from 7 stumps rosetted, and that from 3 stumps grew normally. At the time of decapitation the stem of each tree was cut into 5 sections, numbered 1 to 5 from base to top. In each instance, section 3 included the original bark-patch inoculum. Sections 1, 3, and 5 each furnished 4 bark patches for indexing—2 from the terminal and 2 from the basal end of each section. These bark patches were grafted on 60 index trees, 2 grafts per tree. No transmission took place from inoculum derived from the 3 inoculated trees whose stumps produced only normal growth or from 2 trees whose sucker growth did rosette. Of the other 5 trees whose sucker growth rosetted, 1 transmitted to 1 index tree that had received inoculum from section 1; 1 transmitted to 1 index tree that had received inoculum from section 3;and 2 transmitted to 1 index tree that had received inoculum from section 5. The fifth tree transmitted to 2 index trees, 1 that received inoculum from section 3 and the other from section 5. Thus, of the 42 index trees representing 7 trees known to have been successfully inoculated, only 6 (14 per cent) contracted the disease.

Sections 2 and 4 and the remaining portions of sections 1 and 5 of each tree that had been decapitated 3 weeks after inoculation were treated at 50°C for 5, 10, 15, or 20 minutes. After treatment, bark-patch grafts were made on 130 index trees, all of which remained normal.

Record-breaking heat (28 days in which temperatures reached 37.8°C or higher; 340 hours at 35° or higher) and drought that summer—1954—resulted in poor growth of the index trees.

Experiment 6, incubation-stage trees with nurse trees grafted to tops.—In September, 1955, three-year-old *P. angustifolia* seedlings were each inoculated with 2 or 3 dormant buds from normal portions of partially rosetted peach or plum trees. Of 90 trees inoculated, only a few had expressed rosette symptoms before leaf fall. These 90 trees were lifted in January, 1956, and 77 were heat treated. Soon after treatment, one or more branches of each treated tree were grafted to a branch of a healthy peach nurse tree (Fig. 1). Each of the inoculated control trees was similarly grafted. The pairs of united trees were planted in the orchard.

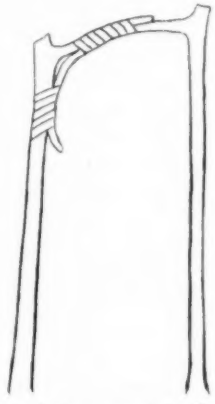

FIG. 1. Grafting technique used in Experiment 6: nurse tree on right; heat-treated tree on left.

In some instances, a branch of a treated tree grafted to a branch of a nurse tree remained alive long after the remainder of the treated tree had died. In others, the nurse tree developed rosette symptoms although the treated tree died before making sufficient new growth to reveal by its own behavior the effects of the treatment on the virus. In a few instances, the treated tree expressed rosette symptoms in new shoots from the base of the tree, whereas the top of the tree died and the attached nurse tree remained normal. Of 13 control trees, 2 remained normal because of failure of inoculation. Of 77 treated trees, 7 remained normal, perhaps for the same reason. For the 6 temperature treatments—40°, 45°, 48°, 50°, 52°, and 55°C—the longest periods of treatment survived by the virus were 18 hours, 150 minutes, 44 minutes, 24 minutes, 15 minutes, and 4 minutes, respectively. With the exception of the 48° treatment, each of these periods was the longest employed in their particular temperature series (Table 2).

TABLE 2.—*Effect of heat treatments on further expression of symptoms in incubation-stage rosette-inoculated Prunus angustifolia seedling trees, each grafted to a peach nurse tree after treatment*

Treatment	Number of trees treated	Expressed further symptoms and/or transmitted	Died without transmitting	Produced normal growth, lived
40°C				
3 hr.	2	2	0	0
4	2	0	2	0
5	2	0	2	0
6	2	1	1	0
8	2	0	2	0
12	2	0	2	0
15	2	1	1	0
18	2	2	0	0
45°C				
75 min.	2	2	0	0
90	2	0	2	0
120	2	1	0	1
150	2	2	0	0
48°C				
28	3	0	2	1
32	3	3	0	0
36	3	3	0	0
40	3	3	0	0
44	3	1	2	0
48	3	0	3	0
52	3	0	1	2
50°C				
8	2	0	1	1
12	2	2	0	0
16	2	1	1	0
20	2	0	2	0
24	2	1	0	1
52°C				
6	2	1	0	1
8	2	2	0	0
9	2	1	1	0
10	2	1	1	0
12	2	1	1	0
15	2	1	1	0
55°C				
120 sec.	2	2	0	0
150	2	1	1	0
180	2	1	1	0
210	2	2	0	0
240	2	2	0	0
Control (untreated)	13	7	4	2

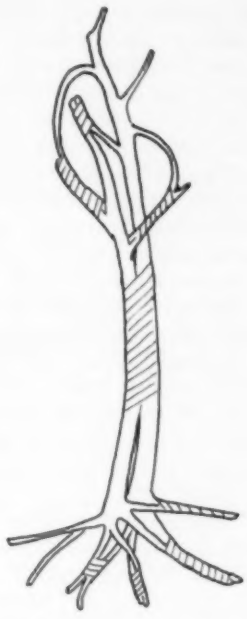

FIG. 2. Grafting technique used in Experiment 7: nurse tree on right; heat-treated tree on left.

Experiment 7, entire trees with nurse trees grafted to roots, stems, and trunk.—1) *Thermal death time at 50°C.*—Peach trees that had been inoculated with bark-patch grafts in April, 1956, and had produced one or more rosetted branches were heat treated at 50°C in December, 1956. On the latter date their trunk diameters ranged from 30 to 66 mm. Treatments were for 20, 30, 35, 40, 60, or 90 minutes. Three trees were treated for each period, and 3 untreated trees served as controls. Each tree was then grafted to a *P. angustifolia* nurse tree by unions of roots, trunk, and stem wherever contacts could be made (Fig. 2). The paired trees were planted in the orchard. All the peach trees died, except for 1 or more branches nursed by graftage to a plum branch on each of 6 trees, and all such peach branches produced rosetted growth. These were on 2 control trees, 1 tree treated 30 minutes, 2 treated 40 minutes, and 1 treated 60 minutes. The branch on the tree treated 60 minutes survived into July and produced 6 inches of rosetted growth.

P. angustifolia trees served well as nurse trees because of the pliability of the small branches employed in grafting, but the incubation period of rosette is longer in *P. angustifolia* than in peach. Although *P. angustifolia* trees usually do not show symptoms in the year they are inoculated, the trees in this experiment were frequently and heavily pruned to force symptom expression. By fall, 6 nurse trees had unquestionable symptoms and many more had questionable symptoms.

In the 60-minute group, 1 nurse tree became fully rosetted and 1 partially so.

2) *Thermal death temperature in 10 minutes.*—Two-year-old plum trees of the Methley variety were inoculated with PRV by bark-patch grafts in September, 1956. In December, at which time their trunk diameters ranged from 27 to 38 mm, they were treated at 50°, 52°, 54°, 56°, or 58°C for 10 minutes. Four trees were used for each treatment. Each was then grafted to a *P. angustifolia* nurse tree, as in the experiment just described. Rosette-inoculated Methley trees in the incubation stage withstood heat treatment much better than did rosetted peach trees, but all those treated at 56° or 58° died. By fall, 1957, rosette symptoms had developed in at least the Methley tree of each pair in the 50° group, 2 of 4 in the 52° group, 3 of 4 in the 54° group, and 1 *P. angustifolia* nurse tree in the 56° group; as stated previously, all Methley trees in the last group died.

Discussion.—The difference is rather great between the thermal death time reported earlier for PRV—10 minutes at 50°—and the more than 60 minutes reported here. The discrepancy may be due to the occurrence of strains of virus that differ in heat stability, or it may be due to the techniques employed. The presence of non-rosetted test trees in some experiments reported here may have resulted from failure of inoculation in some instances and death of virus-invaded tissues in other instances.

Virus strains.—At Fort Valley, 10 sources of PRV were employed, including naturally infected peach, *P. angustifolia*, and *P. injucunda* Small.[3] In some experiments each replicate of a treatment represented a different source. With respect to heat tolerance, no differing strains of PRV were found. However, all infected material came from Peach or Crawford Counties, Ga., within 60 miles of where Smith (6) described peach rosette. The possibility was not investigated of the occurrence in distant localities of strains varying in heat tolerance.

Inoculations.—Some viruses that affect peach, such as peach mosaic virus (2) and elm mosaic virus (1), have been reported as so easily transmissible that even slight graft unions effect transmission, whereas transmission of PRV requires a substantial graft union. Kunkel (5) determined that a bud-contact period of 8–14 days was usually necessary for PRV transmission from peach to peach under the conditions of his experiment. At Fort Valley, with less favorable conditions for growth and lower humidity under orchard conditions, a graft union of 14 days between rosetted and normal trees did not always effect transmission, although permanent unions always did so. Failure to secure successful inoculation with bark-patch grafts may perhaps result from the death of the tissues containing the virus before the graft-contact period is sufficiently long.

In peach, PRV causes all buds to initiate growth, with the result that no buds are dormant on the rosetted portions of peach trees. More than a thousand inoculations with PRV have been made in various experiments at Fort Valley. In some, such as Experiment 6, dormant buds were taken from partially rosetted trees, with a high per cent of transmission usually resulting from those taken close to rosetted branches. But most inoculations were made with bark-patch grafts because their survival has usually been longer than that of buds. In spring and early summer under humid conditions in the screen house, nearly 100 per cent transmission was regularly obtained with bark patches or slightly-growing buds grafted to small heavily pruned peach trees. Even in the screen house, transmission never approached 100 per cent with test trees 3 years old or older. In midsummer, bark-patch grafts, 2 or 3 per tree, on 2-year-old peach trees growing out of doors averaged about 50 per cent transmission.

In the heat-treatment experiments, inoculated trees were not always of a favorable age, in a favorable location, or inoculated at a favorable time to anticipate 100 per cent transmission. In Experiment 2, inoculations were made in October out of doors. Transmission was 71 per cent, assuming that PRV was not inactivated by any of the treatments. In Experiment 4, part 2, inoculations were made out of doors in April. Transmission was 50 per cent for inoculum from trees treated less than 15 minutes at 50°C and 6 per cent for that from trees treated 15 minutes or longer. This difference is not necessarily due to inactivation of the virus by heat. The root bark of all source trees appeared alive and normal, but it is possible that varying degrees of vigor obtained in their roots, depending on the duration of the heat treatment. Thus, root bark from trees treated 4 minutes at 50°C may have made longer graft contact with test trees than did root bark from trees treated 15 minutes or longer.

In Experiment 5, bark-patch grafts with material from successfully inoculated incubation-stage trees, 2 per test tree, gave only 14 per cent transmission. The weather was unusually unfavorable, but other conditions were favorable: the test trees were small and heavily pruned, and the inoculations were made in late April. From outward appearances the bark patches seemed to survive for several weeks. Possibly the inoculum did not contain PRV in high enough titer to effect transmission.

The results of Experiment 4 seem to indicate that the thermal death time of PRV is less than 90 minutes at 45°, about 18 minutes at 50°, and about 2 minutes or slightly longer at 55°C. The logarithms of these points fall in a nearly straight line. But the same virus source, used again (with others) in Experiment 6, proved capable of withstanding greater heat. In Experiment 4, 2-year-old peach seedlings crowded in a nursery row were inoculated with PRV in April, 1953, without much pruning. Limited growth resulted in tardy expression of symptoms in many trees and must also have resulted in slow movement

[3] This species of plum has not been reported previously as a host of peach rosette.

of the virus. Only trees with symptoms of rosette in one or more branches before leaf fall were used for heat treatments. Some trees were fully rosetted. During the period of shock following heat treatment, no appreciable movement of the virus would be anticipated, because virus movement appears to be related to growth of the host. For the most part the buds on the treated trees unfolded in the spring, but those on formerly rosetted branches soon died, and the branches themselves died and die-back progressed. On many trees the greater part of the tree top was dead by early April, 1954. Some trees developed rosetted shoots on large branches, trunk, or crown. Fewer developed normal growth, but some of these survived, eventually producing extensive normal growth, and remained normal to the end of the experiment, in 1956. If a treatment resulted in the death of all rosetted portions of a tree, and die-back progressed well below the rosetted portions, the results might be the same as though the tree top had been lopped off well below the lowest point of symptom expression. Thus, all tissues invaded by the virus may have died as a result of the treatment, leaving the tree virus-free. For any given temperature, brief treatments resulted in death of the trees—from the combined effect of heat injury and active virus. Longer treatments resulted in the death of all virus-invaded tissues, with survival of some trees. Still longer treatments resulted in the death of the tree, from heat injury.

The results of Experiment 7 are incomplete. Final readings cannot be made until late summer, 1958, but the point is already made that the thermal death time of PRV exceeds that of its hosts. One branch, grafted to a nurse tree, developed 6 inches of rosetted growth after treatment for 60 minutes at 50°, even though the remainder of the treated tree died. Another tree given the same treatment died without expression of further symptoms, but transmitted to its nurse tree. The same is true for one tree treated 10 minutes at 56°.

Size of trees.—Rather large trees were employed in some of these experiments. The larger the tree, the more likely it was to survive heat treatment. However, the effect of heat treatment was not measured to any great depth into the tree trunk. Some trees that failed to survive heat treatment transmitted PRV to their nurse trees. Unless it is assumed that the virus is present in xylem as well as phloem and that movement of the virus from xylem to phloem took place in these dying trees, the virus transmitted must have been in the vicinity of the cambium, at no great distance from the bark surface. The time required for heat to penetrate that distance into a trunk 60 mm in diameter should not be appreciably greater than the time required for heat to penetrate the same distance into a small bud stick such as used in most earlier experiments involving heat treatments of stone fruit viruses.

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BOOK LISTING

Went, F. W. 1957. **The experimental control of plant growth.** Chronica Botanica Co., Waltham, Mass. 343 p., 25 pl. Price $8.50.

This book is an account of the work done in the Clark greenhouses and the Earhart Plant Research Laboratory at the California Institute of Technology. Part I deals with their construction and operation, Part II with the climatic response of individual plants. Individual chapters deal with the following plants or groups of plants: the tomato and other solanaceae, the pea, the broad bean, the strawberry, miscellaneous cultivated plants, grasses, trees and shrubs, miscellaneous wild plants, and chaparral plants. Part III is devoted to a general discussion of genotypic and phenotypic variability, growth analyses, plant climatology, ecology, germination, photosynthesis, nutrition, water relations, methods for determining water loss, smog and periodicity, and biochemistry.

Use of Gomphrena globosa in Quantitative Work with Red Clover Vein Mosaic Virus. C. S. RAMAMURTHI,[1] A. F. ROSS, AND D. A. ROBERTS. Red clover vein mosaic virus[2] (*Marmor trifolii* Holmes) probably is the same as Wisconsin pea stunt virus, and its reported hosts are in the Leguminosae.[3] Recently, however, it was observed that local necrotic lesions developed about a week after Carborundum-dusted leaves of globe amaranth (*Gomphrena globosa* L.) had been inoculated mechanically with juice from red clover plants that showed characteristic vein mosaic symptoms. Lesions were large, attaining a diameter of ¼ in., and consisted of tan necrotic centers with anthocyanescent margins (Fig. 1). No other report of a local lesion host for this virus has been found. The suitability of globe amaranth for quantitative work with red clover vein mosaic virus was tested.

Several diseased red clover plants taken from a field near Ithaca, N. Y., were transplanted to sterilized soil in 12-in. clay pots and kept in the greenhouse as source plants. There was no evidence of infection by viruses other than the red clover vein mosaic virus. Leaves from these plants were ground in a mortar, and the juice was strained through cheesecloth to remove large particles of leaf tissue. Inocula, prepared by dilution of this crude juice with either water or 0.01 M phosphate buffer (pH 7.0), were applied with cheesecloth pads to Carborundum-dusted leaves of test plants.

Before reliable tests could be made of the suitability of globe amaranth for biological assays, it was necessary to determine the range of dilution of juice over which satisfactory assays might be made. In each of 2 preliminary tests involving the use of 6 plants that had been trimmed to 3 pairs of leaves each, a few lesions were produced by some inocula diluted as much as 10^{-5}. In these 2 tests, lesion counts for juice diluted with water were practically the same as those for juice diluted with phosphate buffer. In a third comparable experiment, dilution with buffer resulted in 2–3 times as many lesions as did dilution with water; however, since the 2 sets of plants differed in age, it is concluded that the differences in lesion counts obtained reflect variations between the 2 different age groups of plants rather than a difference between buffer and water as diluents. In all of these tests, more lesions were induced by inocula diluted $10^{-0.5}$ and 10^{-1} than by crude juice, which apparently contains an inhibitor. Successively fewer lesions resulted with each dilution greater than 10^{-1}.

[1] Assistant Plant Pathologist, Central Potato Research Institute, Simla, India.

[2] Osborn, H. T. 1937. Vein-mosaic virus of red clover. Phytopathology 27: 1051-1058.

[3] Hagedorn, D. J., and E. W. Hanson. 1951. A comparative study of the viruses causing Wisconsin pea stunt and red clover vein mosaic. Phytopathology 41: 813-819.

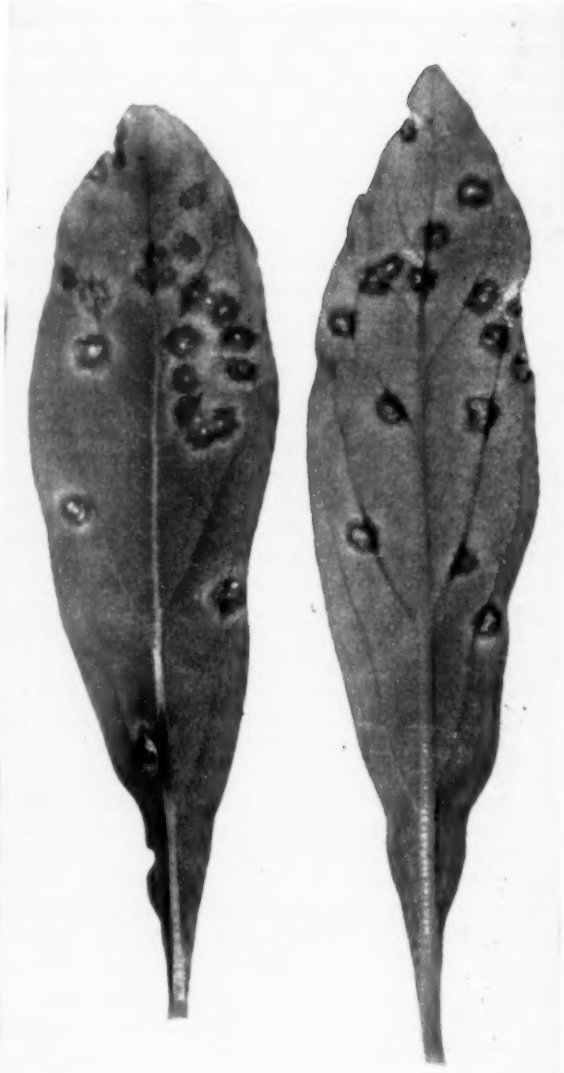

FIG. 1. Local lesions produced on leaves of *Gomphrena globosa* by red clover vein mosaic virus. (Photograph by H. H. Lyon, Jr.)

The dilution curve obtained from results of a fourth test (Fig. 2) was typical of those that were plotted from results of previous tests. When plotted on semilogarithmic paper, the lesion counts for dilutions of 10^{-1}, 10^{-2}, and $10^{-2.5}$ very nearly formed a straight line with a slope of approximately minus 1. These results indicate that satisfactory quantitative assays of red clover vein mosaic virus in juice of infected red clover plants should be possible on this test plant provided the juice is diluted within the range of 10^{-2}–$10^{-2.5}$. A water dilution of 10^{-2} was used as a standard in subsequent experiments.

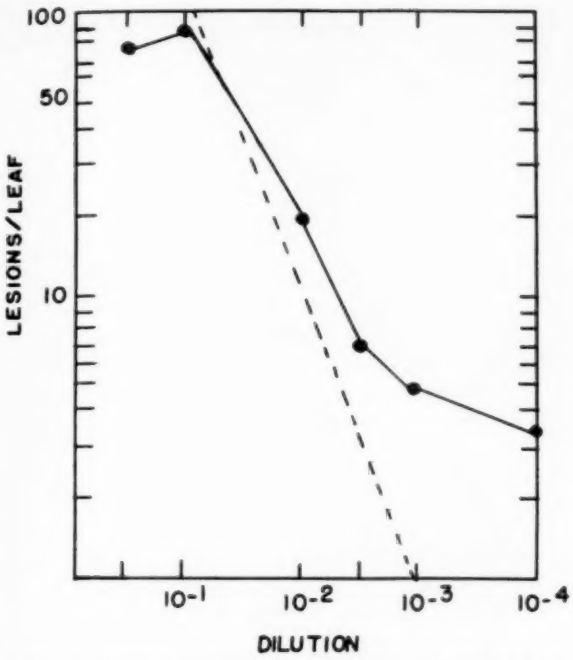

FIG. 2. Average number of local lesions per leaf of *Gomphrena globosa* inoculated with different dilutions of juice from leaves of red clover infected with red clover vein mosaic virus. Each number is the average of lesion counts from 12 leaves. The broken line represents a slope of minus 1.

In each of 5 experiments, the 10^{-2} dilution was compared with a somewhat smaller or with a somewhat greater dilution on 24 pairs of opposite leaves on 12 plants. Dilutions ranged from 1.25×10^{-2} to 0.8×10^{-2}. In 2 experiments the difference between the number of lesions induced by dilutions of 10^{-2} and that induced by dilutions of 1.11×10^{-2} or 0.87×10^{-2} were not statistically significant. In the 3 other tests, however, inoculations with dilutions of 1.18×10^{-2} and 1.25×10^{-2} resulted in significantly more lesions than did inoculation with a dilution of 10^{-2}. Further, significantly fewer lesions formed on leaves inoculated with dilutions of 0.833×10^{-2} and 0.8×10^{-2} than on those inoculated with juice diluted 10^{-2}. All of these differences were statistically significant where P was less than 0.01, as determined by the "t" test for significance. In 1 of these 3 tests, an average of 34 lesions/leaf was produced by inoculum diluted 10^{-2}, and an average of 46 lesions/leaf was produced by inoculum diluted 1.25×10^{-2}. In another test, 55 lesions/leaf occurred when the inoculum had been diluted 10^{-2}, and 37 lesions/leaf occurred when the inoculum had been diluted 0.8×10^{-2}. The average count/leaf for the 10^{-2} dilution was 7, 10, and 15 in 3 other tests. This variability probably resulted from variations among different groups of plants and also from fluctuating greenhouse temperatures in the summer months, when these experiments were conducted.

Results of the tests reported here indicate that satisfactory quantitative assays of vein mosaic virus from red clover can be made with globe amaranth. Differences in infectivity as small as 20 per cent were detected consistently with aqueous dilutions near 10^{-2}. —Plant Pathology Department, Cornell University, Ithaca, New York.

Stereum sanguinolentum on Living Norway Spruce Following Pruning. JOHN H. RISLEY AND SAVEL B. SILVERBORG.[1] *Stereum sanguinolentum* Alb. & Schw. ex Fr., a common destroyer of coniferous slash in North America, also causes a serious heartrot in living balsam firs, *Abies balsamea* (L.) Mill.[2, 3, 4] The fungus has been reported to cause rapid decay of the heartwood of eastern white pine, entering through pruned branches larger than 2 in. in diameter.[5]

In 1956, *S. sanguinolentum* was found causing extensive heartrot in Norway spruce (*Picea abies* (L.) Karst.) associated with pruning wounds, in a plantation near the town of Alder Creek, in central New York. The plantation, of 27 acres, was established in 1932 on a southeast slope at an elevation of 1600 ft. The soil, a glacial till high in dark-colored shale, consists of a medium-textured upper part that is strongly acid and a clayey impervious layer, 15–20 in. down, that is slightly acid to neutral. In 1956 the trees averaged 26 ft. in height and 4 in. in diameter at breast height. In 1951, 12-ft. access ways were cut through the plantation by removing 1 row of trees. The edge trees in these cleared strips were pruned with an axe to a height of about 6 ft. In the pruning process many of the branches, averaging ½–1 in. thick, were broken off, leaving ragged branch stubs several inches long. Other branches were severed so close to the main stem that sapwood of the stem was exposed.

Five years after the trees were pruned, *S. sanguinolentum* was found fruiting abundantly, both on the branch stubs (Fig. 1) and on exposed sapwood. Six affected trees were felled and the extent of decay measured. The total vertical decay originating from the pruning wounds ranged from 6.5 to 11.0 ft. If infection took place shortly after pruning and if the rate of decay was uniform, the average decay rate was 1.3–2.2 ft. per year. This is exceptionally rapid decay for heartrot organisms. Both the heartwood and sapwood

[1] Respectively, Forest Biologist, New York Conservation Department, and Research Associate in Forest Pathology, State University College of Forestry at Syracuse University.

[2] McCallum, A. W. 1928. Studies in forest pathology. 1. Decays in balsam fir (Abies balsamea Mill.). Can. Dept. Agr. Bull. 104. 25 p.

[3] Spaulding, P., and J. R. Hansbrough. 1944. Decay in balsam fir in New England and New York. U. S. Dept. Agr. Tech. Bull. 872. 30 p.

[4] Spaulding, P., G. H. Hepting, and H. J. MacAloney. 1932. Investigations in decays of balsam fir. 1. Gale River Experimental Forest, New Hampshire. Northeast. Forest Expt. Sta. Tech. Note 11. 3 p.

[5] Spaulding, P., H. J. MacAloney, and A. C. Cline. 1935. Stereum sanguinolentum a dangerous fungus in pruning wounds on northern white pine. Northeast. Forest Expt. Sta. Tech. Note 19. 2 p.

FIG. 1.—*Stereum sanguinolentum* fruiting at a pruned branch node.

FIG. 2.—Cross sections of Norway spruce directly above a pruned branch node. A) Decay in the heartwood following entrance of *Stereum sanguinolentum* through a pruned branch. B) Decay following entrance of *S. sanguinolentum* through exposed sapwood.

(Fig. 2) were decayed in some trees, resulting in their death. Cultures from decayed heartwood were confirmed as *S. sanguinolentum*,[6] and the sporophores agreed with the description by Burt.[7] Although *S. sanguinolentum* has been reported to cause serious decay both in living trees and in stored pulpwood of Norway spruce in Scandinavia,[5] this is believed to be the first report of it in this country associated with serious heartrot of living Norway spruce following pruning.

The following recommendations are suggested for keeping heartrot losses at a minimum: 1) branches should be pruned flush with the main trunk to promote rapid callousing and 2) trunk wounds that expose sapwood should be avoided.—New York State Conservation Department, Albany, New York, and State University College of Forestry, at Syracuse University, Syracuse, New York

Production of Cellulolytic Enzymes by Sclerotium Rolfsii. AKHTAR HUSAIN. *Sclerotium rolfsii* Sacc., the causal agent of southern stem rot or blight, is a common facultative parasite of various crop plants in warmer regions of the world. The most characteristic effect of this organism is the rotting of affected plant tissues. Pectic enzymes have been considered as the principal agent involved in the tissue breakdown caused by this fungus as well as certain other fungi that produce soft rot diseases.[1]

Since *S. rolfsii* produces propectinase[2] and a depolymerase[1], it can be assumed that these enzymes play an important role in the separation of host cells that occurs following infection. In affected tissues of plants invaded by *S. rolfsii*, however, a pronounced breakdown and collapse of cell walls also occurs, indicating that cellulolytic enzymes as well as pectic enzymes may be involved. In a review of the literature, reports of the production of cellulolytic enzymes by this fungus were not found. This suggested the advisability of the present investigation of the cellulolytic enzymes of *S. rolfsii* and of the possible relationship of these enzymes

[6] Cultural identification was made by Dr. Mildred Nobles, Senior Mycologist, Botany and Plant Pathology Laboratory, Department of Agriculture, Science Service, Ottawa, Canada.

[7] Burt, E. A. 1920. Thelephoraceae of North America. XII Stereum. Ann. Missouri Botan. Garden 7: 81-248.

[1] Wood, R. K. S. 1955. Pectic enzymes secreted by pathogens and their role in plant infection. In Mechanisms of microbial pathogenicity, 5th Symposium Soc. Gen. Microbiol., Cambridge Univ. Press. p. 263-293.

[2] Milthorpe, F. H. 1941. Studies on Corticium rolfsii (Sacc.) Curzi (Sclerotium rolfsii Sacc.). I. Cultural characters and perfect stage. II. Mechanism of parasitism. Proc. Linnean Soc. N. S. Wales 66: 65-75.

to pathogenesis. A preliminary report of this study has been presented.[3]

The liquid medium used to obtain enzyme samples was a modified Richard's solution containing 0.1 per cent yeast extract with either 3 per cent dextrose or 2 per cent cellulose as sources of carbon. Filter paper pulp and carboxymethylcellulose (CMC, 70 Prem. Medium viscosity, Hercules Powder Co.) were used as sources of cellulose. The culture of *S. rolfsii* used was a highly pathogenic isolate from peanut (*Arachis hypogaea* L.) that was obtained from Dr. W. E. Cooper. Culture filtrates for enzyme assays were prepared by growing the fungus at 30°C in still cultures in 250-ml Erlenmeyer flasks containing 50 ml of the liquid medium. After 7 to 10 days of growth, the medium was freed of mycelium by filtration and the culture filtrate was used as the enzyme source. When stored, the filtrate was covered with a layer of toluene and held at 5°C.

Assays for cellulase were carried out by the cup-plate method of Dingle et al[4] and by measurement of the loss in viscosity of CMC.[5, 6] The latter procedure determines the activity of the Cx enzyme, an enzyme that can break the β-1,4-glucosidic linkage and hydrolyze a straight chain of anhydroglucose units to form cellobiose. Viscosity was measured in size 300 Fenske-Ostwald viscometers at 30° ± 0.1°C in a constant-temperature waterbath. A 1.2 per cent solution of CMC containing citrate buffer at pH 4.0 was used as the enzyme substrate.

To determine whether the Cx enzyme is formed *in vivo*, tomato (*Lycopersicon esculentum* Mill.) fruits in the pink stage of ripening were inoculated with mycelial discs from a young culture of *S. rolfsii* growing on potato-dextrose agar plates. The fruits were surface-sterilized prior to inoculation by dipping in a 1:1000 solution of mercuric chloride for 5 minutes. After inoculation the fruits were placed in sterilized glass jars with cotton plugs. After 2–3 days, the inoculated fruits were homogenized in a Waring Blendor and the juice expressed by squeezing through cheesecloth. The expressed juice was centrifuged at 5000 rpm for 1 hour, and the supernatant liquid was used for enzyme assay. Juice of noninoculated tomato fruits was obtained in a similar manner and also assayed for enzyme activity. The noninoculated fruits were wounded in a manner similar to that followed in the inoculation procedure.

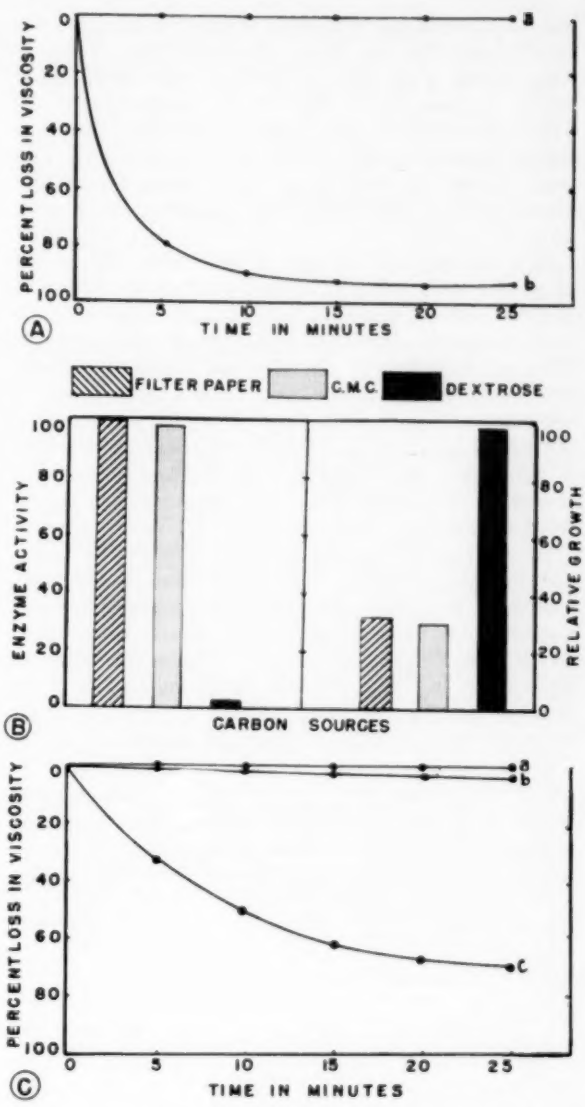

FIG. 1. A) Rate of loss in viscosity of 1.2 per cent solution of carboxymethylcellulose following addition of a) heated and b) nonheated culture filtrate containing cellulase (Cx) of *Sclerotium rolfsii*; B) comparative levels of cellulase (Cx) activity and mycelial growth of *S. rolfsii* in liquid media containing either filter paper, CMC, or dextrose as a carbon source; C) rate of loss in viscosity of 1.2 per cent solution of CMC following addition of extracts from tomato fruits: a) extracts from healthy fruits, b) heated, and c) nonheated extracts from infected fruits.

When enzyme activity was measured by the cup-plate method, the cellulolytic activity of the culture filtrate compared favorably with that of a 1 per cent solution of a commercial cellulase (Enzyme 19-AP, Rohm and Haas Co.). A zone of 2.2 cm was produced by the culture filtrate in comparison to a zone of 2.6 cm produced by the commercial cellulase solution.

Although growth of the fungus was excellent in the medium containing dextrose as the source of carbon, cellulase (Cx) activity of this culture filtrate was very

[3] Husain, A. 1957. Production of a cellulolytic enzyme by Sclerotium rolfsii. (Abstr.) Phytopathology 47: 17-18.

[4] Dingle, J., W. W. Reed, and G. L. Solomons. 1953. Enzymatic degradation of pectins and other polysaccharides. II. Application of cup plate method to the estimation of enzymes. J. Sci. Food Agr. 4: 149-155.

[5] Bell, T. A., J. L. Etchells, and I. D. Jones. 1955. A method for testing cucumber salt stock brine for softening activity. U. S. Dept. Agr. ARS 72-5, 15 p.

[6] Levinson, H. S., and E. T. Reese. 1950. Enzymatic hydrolysis of soluble cellulose derivatives as measured by changes in viscosity. J. Gen. Physiol. 33: 601-628.

low. In contrast, although vegetative growth was considerably less in the media containing CMC or filter paper pulp, enzyme activity was significantly higher in these media than in the dextrose medium. The culture filtrates from both of the cellulose-containing media caused rapid loss in viscosity of a CMC solution. No loss in viscosity occurred when the culture filtrate of the CMC medium was boiled for 15 minutes (Fig. 1, A).

In a second series of tests to determine the amount of growth with different carbon sources in relation to enzyme activity, mycelial weights and enzyme levels were estimated after 10 days of growth of the fungus. Fifty per cent loss in viscosity was taken as an arbitrary unit, and the relative enzymatic activity of different culture filtrates was expressed as the reciprocal of the time requisite for a 50 per cent loss in viscosity. A more than 30-fold increase in enzyme activity occurred when either CMC or filter paper was substituted for dextrose (Fig.1, B).

In experiments to determine the effect of *S. rolfsii* on filter paper cellulose, strips of filter paper were placed in test tubes containing the mineral nutrient medium plus yeast extract and mycelial discs of *S. rolfsii* were added to the tubes. The paper strips in those tubes to which mycelial discs were added were disintegrated by the fungus within 2 weeks, whereas strips in sterile test tubes remained intact.

Following inoculation of the tomato fruits with *S. rolfsii*, the fruits decayed rapidly. In the enzyme assays on juices from noninoculated and inoculated tomato fruits, the Cx enzyme was absent in healthy fruits but present in inoculated fruits (Fig. 1, C). Similar results were obtained in 2 additional tests. No organism other than *S. rolfsii* was detected in isolations made from both diseased and healthy fruits, indicating that the enzyme present in these fruits was formed by *S. rolfsii*.

On the basis of the above results, it can be concluded that *S. rolfsii* produces the cellulolytic enzyme designated by Reese et al[7] as the Cx enzyme. Since this enzyme is also produced in the diseased host, it can be assumed that the cellulase produced by this fungus is probably involved in the breakdown of cellulosic components of cell walls of plant tissues invaded by this organism. This assumption is supported by the evidence that the fungus can also digest filter paper cellulose. Digestion of filter paper cellulose indicates that the C_1 enzyme[6] (a postulated enzyme that converts native cellulose into simple straight chains of anhydroglucose residues) also is produced.

The production of cellulolytic enzymes by *S. rolfsii* is significant since the role of these enzymes in pathogenesis by those fungi that cause soft rot diseases has rarely been emphasized. Wood[1] has suggested that pectic enzymes in association with other enzymes bring about dissolution of cell walls in tissues invaded by certain plant pathogens. These results indicate that cellulolytic enzymes in addition to pectic enzymes are involved in the breakdown of cell wall components in pathogenesis by *S. rolfsii*.—Plant Pathology, North Carolina State College, Raleigh, North Carolina.

[7] Reese, E. T., R. H. G. Siu, and H. S. Levinson. 1950. The biological degradation of soluble cellulose derivatives and its relation to mechanism of cellulose hydrolysis. J. Bacteriol. 59: 485-497.

REPORT AND ABSTRACTS OF THE 1958 ANNUAL MEETING OF THE SOUTHERN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The Southern Division of the American Phytopathological Society held its annual meeting in conjunction with the 55th annual convention of the Association of Southern Agricultural Workers, at Little Rock, Arkansas, February 3-5, 1958. About 100 plant pathologists and guests attended; 38 papers were presented.

The following officers were elected at the business meeting, February 3: *President*, G. B. Lucas; *Vice-President*, E. M. Cralley. Councilor for the Division is D. E. Ellis.

W. J. Martin, *Secretary-Treasurer*

ABSTRACTS OF PAPERS PRESENTED AT THE MEETING

Control of cercospora leafspot of Photinia serrulata with fungicides. Anzalone, Louis, Jr., and A. G. Plakidas. The fungicides used were captan, ferbam, tri-basic copper sulfate and copper-A at 3 lb./100 gal., and 4-4-50 Bordeaux mixture. Triton X-100 was used at the rate of 1 pt./100 gal. in all sprays. In 1 series of greenhouse tests, the fungicides were applied and allowed to dry, after which the plants were immediately inoculated. In another series, the fungicides were applied and allowed to dry, then the plants were subjected to 30 minutes of artificial rain each day for 6 days before inoculation. The percentage of leaf area infected in the former and latter test series, respectively, was: for the noninoculated check, 0, 0; inoculated check, 66, 74; triton, 59, 83; tri-base copper sulfate, 34, 55; copper-A, 25, 58; captan, 18, 45; Bordeaux, 5, 8; and ferbam, 5, 48. In both greenhouse tests the plants were kept moist by a water mist for several days after inoculation. In field tests, all leaves of 3-year-old plants were removed 3 weeks before the first of 3 sprayings, applied at 2-week intervals. Surrounding diseased plants provided natural inoculum. Leaf infection percentages were 74, 76, .6, 21, 44, .9, and 27, respectively, for the check, triton, tri-basic copper sulfate, copper-A, captan, Bordeaux, and ferbam. All copper fungicides caused some phytotoxicity.

Effect of cutting roots on the incidence of Fusarium wilt of cotton, tomatoes, cowpeas, and other plants. Armstrong, G. M., and J. K. Armstrong. Wilt-susceptible varieties were grown in 2-gal. glazed pots containing steamed sand to which a mineral nutrient solution was added daily. In the first series of experiments, young plants were inoculated by cutting roots on 1 side and pouring a liquid inoculum on the cut roots. When each plant showed moderate to severe external symptoms of wilt, the date was recorded, and it was removed from the pot. These pots were used immediately in the second series, in which 500 ml of inoculum was mixed in the top 2 in. of sand, the seed planted, and the roots left undisturbed. When each plant reached a comparable stage of wilting, as above, the stem was cut off about 2 in. above the soil line, but the roots were not disturbed. The percentage of plants that showed external symptoms of wilt, and the average number of days required for the symptoms to develop were recorded. For example, the average per cent of wilting of Rowden cotton increased from 82.5 (roots undisturbed) to 98.3 (roots cut). The corresponding figures for susceptible tomatoes were 82.4 and 98.5. Respectively, the average number of days for wilting of cotton was 46.7 and 14.8; for tomatoes, 54.8 and 17.6.

Reaction of red and white clover introductions to root-knot nematodes. Bain, Douglas C. Thirteen white and 48 red clover introductions were tested for reaction to *Meloidogyne incognita*, *M. incognita acrita*, *M. arenaria*, and *M. javanica*. Readings were made about 70 days after planting in infested soil. Degree of infection among plants of individual introductions ranged from very light to severe and killing. In many instances, particularly in certain white clovers, plants apparently free of root-knot were classed as having light infection because of the possibility of overlooking very small nematode galls. With certain exceptions, both red and white clover were moderately to severely infected by all 4 species of nematodes. Check commercial varieties were severely infected. Four of the white clovers, plants apparently free of root-knot were *incognita acrita*, 1 by *M. javanica*, but none by the other 2 species. Similar results were obtained in a second test involving *M. incognita* and *M. incognita acrita* with the 4 introductions indicated above. Among the red clovers, 1 and 2 introductions, respectively, were lightly infected by *M. arenaria* and *M. javanica*. The results suggest the possibility of selecting white clovers resistant to *M. incognita acrita* and *M. javanica*, and red clovers resistant to *M. arenaria* and *M. javanica*.

Thermotherapy for nemas of ornamental plants. Birchfield, Wray, and H. M. van Pelt. Among the most common nemas associated with ornamental plants in the Apopka area of Florida are *Helicotylenchus multicinctus* and *Meloidogyne incognita*. Thermotherapy was used to obtain propagating stock relatively free of these pests. To find a suitable treatment the nemas were subjected to 50°C under 2 conditions: treatment of free-swimming nemas in glass vials; and treatment of ornamental plants harboring the nemas. A susceptible tomato was used to assay the effects of the treatment in both cases. Galls were not observed on tomato exposed to *M. incognita* treated 4, 5, and 10 minutes. Similarly, *H. multicinctus* was not recovered from tomato exposed to those nemas treated for 2½, 5, and 10 minutes at 50°C. Hot water (10 minutes at 50°C) was used as a dip for ornamental plants infected with *M. incognita*. Non-treated ornamental plants served as checks. After treatment the ornamental plants were placed in steam-sterilized soil and the tomato next to them. A few tomatoes showing galls (6 of 300 examined) were found among the treated, whereas many tomato plants showing galls (82 of 282 examined) were found among the checks or nontreated ornamental plants. Ornamentals tolerating the treatment and showing favorable root response were: Boxwood, Caladiums, Chinese Evergreen, Sansevieria, and Gardenia.

The number of days from inoculation to first symptoms, a measure of the degree of resistance to the bacterial blight disease of cotton. Bird, L. S. The B_2 gene for resistance to bacterial blight of cotton, caused by *Xanthomonas malvacearum* (E. F. Sm.) Dows., was the source of resistance used in the study. Thirty-two plants of an F_3 progeny were grown in the greenhouse during the winter of 1948-49. The mean disease grade, mean days from inoculation to first symptoms, and an index obtained by dividing the grade by the number of days were calculated for each plant. The genotype of the greenhouse plants was determined by progeny tests in the field. The correla-

tions, all highly significant, between field progeny mean grade and individual plant mean grade, mean days, and index were respectively +.92, −.81 and +.89. The highly significant correlation between individual plant mean grade and mean days was −.92. The regression of progeny mean grade on individual F_2 plant mean grade, an estimate of heritability, was 0.79. The high association between degree of resistance and days from inoculation to first symptoms indicated that resistance was due to a physiological mechanism that deprived the organism of an adequate source of energy.

Differences in amino acid utilization by Xanthomonas malvacearum races 1 and 2. BLACKMON, CYRIL W. The utilization of 22 L or DL amino acids by *X. malvacearum* races 1 and 2 was tested by growth in nutrient solution and measured as turbidity at 3, 6, and 16 days. Norvaline was the only amino acid utilized by race 1 faster than by race 2. Asparagine, arginine, lysine, proline, cystine, methionine, A-alanine; phenylalanine, tyrosine, isoleucine, and valine were utilized by R-2 at a faster rate than R-1. Threonine, glycine, B-alanine, and histidine were utilized to some extent by R-2 by the sixteenth day, but they were not used at all by R-1. Serine, norleucine, aspartic acid, glutamic acid, and cysteine were not utilized by either race, and appeared to be inhibitory, since some growth occurred in the control, which was Shive's solution with glucose added. In combinations involving valine, leucine, isoleucine, norleucine, threonine, and serine, no real differences appeared between the 2 races. Serine and threonine still completely inhibited growth of both races in all combinations in which they were used. These studies indicate that the amino acid content of cotton leaves, stems, and bolls may be involved in physiological resistance to *X. malvacearum*.

Parasitic nematodes associated with strawberries in Arkansas. DICKERSON, O. J., AND D. A. SLACK. Samples of plants and soil were collected from strawberry fields selected at random in commercial production areas. Plant parasitic nematodes were recovered from samples in various combinations. Frequency of nematode recovery from samples representing 93 fields was: *Pratylenchus coffeae*, 32; *P. penetrans*, 23; *Pratylenchus* sp., 8; *Xiphinema americanum*, 13; *Tylenchorenchus dubius*, 3; *Meloidogyne hapla*, 3; *Aphelenchoides besseyi*, 2; *Helicotylenchus erythrinae*, 1; and *Pratylenchus* sp., 2. In addition, *Aphelenchoides parietinus* and species of the genera *Tylenchus*, *Psilenchus*, and *Aphelenchus* were frequently present. Numbers of nematodes recovered from the samples varied widely. With the exception of *P. coffeae*, nematodes were not found in sufficient numbers to indicate serious damage to commercial strawberry production, and no relationship could be determined between number of nematodes recovered and plant vigor. High populations of *P. coffeae* were recovered consistently in association with black root rot of strawberry plants growing on relatively heavy soil only. Results do not include a special survey for *Aphelenchoides besseyi* (Plant Disease Reptr. 41: 398-401).

A cause of oat yellowing. EARHART, R. W., AND M. H. THOMAS, JR. The chlorotic yellowing and striping of many of the oat plants grown in the Piedmont area of southeastern United States in late March and April of 1957 raised much speculation as to its cause or causes. These striking symptoms had previously been found associated with oat plants growing in soils of high pH (above 6.3) where mineral nutrition had become unbalanced. However, investigations demonstrated that this particular condition was not associated with high pH soil, since, under South Carolina conditions, these particular symptoms were expressed by plants growing in soils of pH 4.9–7.3. Recent work at Clemson has repeatedly demonstrated that a yellowing and striping condition similar to that expressed by oat plants in the field can be induced by planting oats in soil that has been artificially infested with selected single-spore strains of Helminthosporium species that are morphologically similar, if not identical, to *Helminthosporium victoriae* or *H. setariae*.

Preliminary studies on yellow-leaf of oats in Georgia. FARRAR, LUTHER L. During the 1956-57 season an epiphytotic syndrome appeared, primarily on Victorgrain, and to a lesser extent on Arlington, Fulgrain, Redrustproof, and Southland oats. The most striking symptom was a yellowing of leaves. This appears on scattered plants in patches of various sizes, and occasionally over entire fields. Longitudinal yellow striping of leaves occurs between the veins, a yellowing of entire leaves, a yellowing of the central portion of leaves (only the leaf tip and the area anterior to the ligule are green), chlorosis of oat heads, blasting of florets, stunting of plants, and usually a severe reduction in yield. Observations indicate that yellow-leaf is not caused by culturable pathogens, nematodes, minor element deficiencies, soil differences, or agronomic practices. In preliminary transmission studies with aphids, yellow striping of leaves developed in inoculated pots but not in checks. Yellow-leaf occurred in patches similar to those reported for yellow dwarf of cereals. These facts suggest that the malady is caused by a virus or strains of viruses.

Ecological factors affecting the growth of Sclerotium rolfsii. FLADOS, N. D. Linear growth of *S. rolfsii* Sacc. through soils of various textures at various moisture levels and 77° and 90°F was markedly different in sterile and unsterile soils. Growth through unsterile soil of both textures at both temperatures was reduced as soil moisture increased. Growth through sterile soil was better than that found in unsterile soil, but was condiitioned by the interaction of soil moisture, temperature, and texture. The reduction of growth found with increasing soil moisture in unsterile soil was not due to the lower oxygen tensions and/or higher relative humidity levels characteristically found in wetter soils. However, increased relative humidity favors organisms that inhibit the growth of *S. rolfsii*. Decreased oxygen levels also favor the antagonistic organisms. The lower temperature (77°F) was more favorable to the growth of antagonists while 90° favored the *S. rolfsii*. Soils of fine texture, such as heavy clays, probably become limiting to growth through their high moisture-holding capacity, high organic matter content, poor aeration, and small pore spaces. All of these factors apparently influence the activity of *S. rolfsii* indirectly in that they favor organisms antagonistic to it.

Post-plant fumigation for the control of peach root-knot in South Carolina. FOSTER, H. H., AND D. FRED COHOON. Root-knot nematodes (*Meloidogyne* spp.) are present in most sandy soils in South Carolina and could be the major limiting factor in peach production in the newly established orchards in the Allendale-Barnwell area. Soil fumigation experiments were conducted using liquid and granular 1,2-dibromo-3-chloropropane (Nemagon) around root-knot-infected peach trees. Severity of root galling was indexed from 0 to 4 in greenhouse root-knot population studies. Soil from each single-tree plot was collected in the fall of 1956 and 3 tomato plants were grown 30 days in each soil sample at a soil temperature of 80°F. The average number of root-knot galls per tomato plant was estimated as follows: liquid fumigant applied in the fall of 1955 at 8.4 gal. of active ingredient per acre, 8.3 galls; check, 22.6 galls. Liquid fumigant applied in the spring of 1956 at 8 gal. per acre, 0.6 galls; 5 gal., 4.0 galls; 2.5 gal., 66.6 galls; check, 63.3 galls. In recent demonstration plots, the average data for liquid Nemagon 8 gal. per acre and the check plots, respectively, were: shoot length, 63 and 44 in.; increase in trunk circumference, 2.23 and 1.12 in; and root-knot index, 0.6 and 3.0. Liquid Nemagon at the rate of 8 gal. of active ingredient per acre gave excellent root-knot control, and was more effective than granular Nemagon. Recent data indicate that Nemagon may be effective for at least 2 years after fumigation.

Wettable sulfur may act as an eradicant fungicide in the control of peach scab. FOSTER, H. H., AND W. H. RHODES. Peach scab, caused by *Cladosporium carpophilum*, continues to be of economic importance in South Carolina and may be of major importance in the new peach areas. During the past 2 years, wettable sulfur and captan have been evaluated for the control of peach scab in replicated orchard experiments. The per cent of Jerseyland scabby peaches for 1956 and 1957 is as follows: captan (complete spray schedule), 4, 11; wettable sulfur (complete spray schedule), 0.4, 0.3; captan (1st spray omitted), 23, 12; captan (1st and 2nd spray omitted), 42, 29; captan (1st, 2nd, and 3rd spray omitted), 97, 88; wettable sulfur (1st, 2nd, and 3rd spray omitted), 6, 0.2; captan (1st, 2nd, 3rd, and 4th spray omitted), 98, 98; and check, 99, 100. These data indicate that captan was less effective against scab than sulfur, whereas sulfur gave effective control even when the first 3 applications were omitted. Captan with several different adjuvants proved as ineffective as regular captan when the early sprays were omitted.

Pathogenicity of fungi isolated from diseased cotton seedlings in Arkansas. FULTON, N. D., AND KATHARINA BOLLENBACHER. Twenty-two fungus isolates were tested individually for pathogenicity on the seed and seedlings of cotton. Included were *Alternaria* sp., *Ascochyta gossypii*, *Aspergillus flavus*, *A. niger*, *Cladosporium* sp., *Colletotrichum gossypii*, *Curvularia* sp., *Fusarium oxysporum*, *F. moniliforme*, *Helminthosporium* sp., *Penicillium* sp., *Pythium* sp., *Rhizoctonia solani*, *Rhizopus* sp., *Sclerotium bataticola*, *S. rolfsii*, *Thielaviopsis basicola*, *Trichoderma* sp., and *Verticillium albo-atrum*. Tests were made in the greenhouse and laboratory in sand at controlled temperatures from 16–27°C. Preemergence killing was much more prevalent with *R. solani* and *Pythium* sp. than with other fungi. Most isolates were more pathogenic in tests held at a constant temperature of 21°C than in tests held 1 week at 27°C and changed to 16°C. Only *Rhizoctonia solani*, *Pythium* sp., *Colletotrichum gossipii*, *Thielaviopsis basicola*, and *Aspergillus flavus* were highly pathogenic to cotton seedlings. Striking differences in pathogenicity were found among the various isolates of a particular species, except for *Aspergillus flavus*. *Fusarium* spp. were in general only moderately to mildly pathogenic. Other fungi were either non-pathogenic or only mildly so.

The effect of stem rust of varying infection intensities on yield and other factors in wheat. GARRETT, W. N., AND M. C. FUTRELL. Artificial inoculations of stem rust were made in 12 × 12-ft. plots of Bowie wheat at 2-, 4-, and 6-ft. intervals with race 29 of *Puccinia graminis tritici* Erick. and E. Henn. Yield, 1000-kernel weights, and bushel weights were taken on grain from each plot. The greater the number of stem rust infection centers in a wheat field, the greater the reduction in yield per acre, bushel weight, and kernel weight. The rate of development of the rust fungus was rapid during the winter months when a large number of infection centers were present in the plots. Where infection centers were established at 2-ft. intervals the yield of wheat was 2 bushels per acre. With infection centers at 4-ft. intervals the yield was 6 bushels per acre, and at 6-ft. intervals the yield was 8 bushels per acre. Where no infection centers were established and no control used, the yield was 13 bushels per acre. The application of maneb increased the yield, test weight, and kernel weight. Yield per acre was increased from 2 to 21 bushels, and test weight per bushel, from 42 (sample grade) to 60 (number 1 heavy) lb.

Root knot and other nematodes in relation to the development of tobacco black shank. GRAHAM, T. W. Greenhouse soil cultures of 4 nematode species parasitic on tobacco along with sterilized oat-grain cultures of the black shank fungus, *Phytophthora parasitica* var. *nicotianae*, were added to field plots previously fumigated with methyl bromide. Plots were infested with individual nematode cultures in combination with the black shank fungus and with the fungus alone. Seedlings of the moderately resistant variety Dixie Bright 101 were grown in the greenhouse in pots and transplanted to the field without injury to the roots. Moderate to severe black shank developed on plots infested with the combination black shank and root knot, *Meloidogyne incognita* var. *acrita*, during trials in 1955 and 1956. The combination of meadow nematodes, *Pratylenchus brachyurus*, and black shank showed only a trace of black shank although root mutilation was as severe as that of root knot. Plots free of nematodes but infested with black shank and those infested with the combination black shank and spiral nematodes, *Rotylenchus brachyurus*, developed only a trace of black shank. Those infested with the tobacco stunt nematode, *Tylenchorhynchus claytoni*, and the black shank fungus showed very slight root injury but developed slight and moderate black shank, respectively, in 1955 and 1956.

Terraclor for the control of southern blight of peanuts. HARRISON, A. L., AND G. M. WATKINS. Applications of Terraclor (75% pentachloronitrobenzene) as a spray to the crown of Spanish peanuts with each cultivation, for the control of southern blight (*Sclerotium rolfsii*), gave significant increases in yield of nuts at the 1% level in 1956 and 1957. In 1956 Terraclor (total of 15 lb./acre) increased yield of nuts 211 lb. per acre. Zineb (zinc ethylene bisdithiocarbamate) and captan (*N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide) applied in the same manner were ineffective. A randomized split-plot test was conducted in 1957, with 3 moisture levels and 4 fungicide treatments applied as sprays with each of 3 cultivations. Terraclor increased the yield of nuts from 1382 lb. per acre in the check plots to 2047 lb. in the sprayed. There was no significant difference between Terraclor at 18 and 36 lb. per acre. Irrigation increased the yield of nuts but did not significantly affect the effectiveness of Terraclor. Terraclor has given some indication that it may reduce southern blight when mixed in the soil before planting. Captan and zineb were ineffective when applied in this manner.

A field method of testing fungicides for the control of Botrytis rot of strawberries. HORN, NORMAN L. To ensure Botrytis rot of strawberries for testing of fungicides in the field, a sprinkling system was used to maintain high moisture conditions during the day. The apparatus consisted of a series of Teejet TN4W nozzles supported on 12-in. risers set 9 ft. apart on ¾-in. galvanized pipe. The nozzles sprayed the water at right angles to the risers covering an area about 3.5 ft. in diameter. Frequent winds dispersed the water sufficiently to cause a mist across the entire planting. In a preliminary test the average rot of Headliner berries not treated and not sprinkled was 2.0%; not treated but sprinkled, 15.9%; and treated with captan and sprinkled, 2.8%. Six fungicides were tested on 2 varieties of berries under the spraying system: captan plus BTC-824 (alkyldimethylbenzyl-ammonium chloride), Merbam (phenylmercury dimethyldithiocarbamate), captan (control), thiram, Coromerc (*N*-phenylmercuriethylenediamine), and BTC-824 alone. The average % of rots, in the order of the treatments given above, were as follows: on the variety Headliner, 10.0, 25.0, 7.3, 9.3, 6.6, and 38.6; on the variety Konvoy, 35.0, 71.0, 22.0, 21.0, 34.0, and 86.0.

Taxonomy and host range of Alternaria cucumerina. JACKSON, CURTIS R. It is proposed that *Alternaria cucumerina* (E. & E.) Ell. is the valid name for the fungus causing a leaf spot disease of cucurbits and it is suggested that *A. nigrescens* (Pegl.) Neerg. be regarded as a synonym. During the past 2 years the disease has caused severe damage to muskmelons grown in variety plots near Gainesville, Florida. Spring and late-summer plantings became infected during growing periods that were humid and warm, and all vines were defoliated within 6 weeks. Muskmelon leaf

lesions are sub-circular, brown, 0.5–2 cm in diameter, frequently concentrically zoned, and with narrow, chlorotic borders. The pathogen has been found occasionally, forming a black-olivaceous growth on the surface of overripe muskmelons and cucumbers. Conidia from host material average 56.6–86.5 × 18.1–21.4 μ, without beaks, with 5–9 transverse and 2–6 longitudinal septations; average beak dimensions are 106–135 × 2.1 μ. The host range as determined by greenhouse inoculations includes muskmelon, watermelon, cucumber, bur gherkin, balsam apple, and 7 varieties of squash.

Control of tobacco mosaic with an antiviral agent. LUCAS, G. B., AND N. N. WINSTEAD. The effectiveness of an antibiotic, Cytovirin, in inhibiting TMV infection was studied on tomato (Rutgers) and flue-cured tobacco (Dixie Bright 101). In preliminary greenhouse tests, 20 transplants each were atomized with 100 and 500 ppm solutions of crude Cytovirin (8%). About 1 hour later, 1 leaf of each plant was inoculated with TMV. Seventy per cent of the treated plants showed no mosaic symptoms 30 days later, whereas 100% of the control plants showed symptoms within 14 days. In field tests tobacco transplants were sprayed (1 pint/2 sq. yd. plant bed) with partially purified Cytovirin (85%) at concentrations of a) 5, b) 25, or c) 50 ppm 24 hours before pulling the transplants with TMV-contaminated hands. The plants were compared with non-sprayed d) TMV-inoculated and e) non-inoculated transplants. At the end of 30 days, 14, 0, and 6% of the plants in a, b, and c, respectively, exhibited mosaic symptoms; 60% of the plants in d and none of the plants in e were mottled. After 60 days the % of plants showing symptoms had increased to a) 50, b) 20, c) 56, d) 100, and e) 16. Plants in d were about 2 ft. shorter than those in the other treatments.

Inheritance of sex reaction in Cochliobolus nodulosus. LUTTRELL, E. S. Genetic study of *Cochliobolus nodulosus* Luttrell indicates that sex reaction in this presumably hermaphroditic but self-sterile species is determined by a single pair of factors, which are designated MT/mt. Mating-type determinations of 100 cultures from single ascospores isolated at random from crosses between a slow-growing, conidial mt strain and a fast-growing, non-conidial MT strain showed a ratio of 47 MT to 53 mt strains. Factors for sex reaction segregated independently of factors for growth rate and sporulation. MT strains exhibited a tendency toward "maleness" as indicated by the fact that perithecia formed along the juncture of the MT and mt strains or on the mycelium of the mt strain regardless of the relative growth rates or colony types of the isolates mated.

The relationship of root-knot nematodes to the development of Fusarium wilt in alfalfa. MCGUIRE, JAMES M., H. J. WALTERS, AND D. A. SLACK. The effect of the root-knot nematode species, *Meloidogyne hapla*, *M. javanica*, *M. incognita*, *M. arenaria*, and *M. incognita* var. *acrita*, on the development of Fusarium wilt in the Buffalo variety of alfalfa was determined. Treatments of *Meloidogyne* sp. alone, *Meloidogyne* sp. with *Fusarium oxysporum* f. *vasinfectum*, *F. oxysporum* f. *vasinfectum* alone, and check (no treatment) were replicated 4 times for each species of nematode in each of 2 tests. At the end of 4 months the percentage of plants infected with Fusarium wilt in each treatment was as follows: *M. hapla* and *F. oxysporum* f. *vasinfectum*, 95; *M. javanica* and *F. oxysporum* f. *vasinfectum*, 60; *M. incognita* and *F. oxysporum* f. *vasinfectum*, 50; *M. arenaria* and *F. oxysporum* f. *vasinfectum*, 50; *M. incognita* var. *acrita*, 10; *F. oxysporum* f. *vasinfectum*, 15; *Meloidogyne* sp. alone and check, 0. Percentage of dead plants in the above treatments was 40, 5, 10, 4, 0, 3, 0, respectively. The number of plants infected with Fusarium wilt and the severity of infection were in proportion to the severity of root-knot.

The response of root-knot-infected tobacco plants to foliar applications of maleic hydrazide. NUSBAUM, C. J. Interactions between root-knot infections, caused by *Meloidogyne incognita*, and foliar applications of maleic hydrazide (MH) were observed in greenhouse tests. Two groups of tobacco plants were used: 1) transplanted to noninfested soil, and 2) transplanted to soil infested with larvae and egg masses of *M. incognita*. Within each group, MH was applied at 4 rates (0, 5, 10, and 25 mg per plant) and at 7- and 14-day intervals after transplanting. On the basis of terminal growth retardation the response to MH treatment occurred earlier and was more pronounced on plants growing in noninfested soil than on plants in infested soil, indicating that root-knot infections reduced the effect of MH on plant growth. Examination of roots and nematode galls 35 days after transplanting showed that foliar applications of MH inhibited the development of galls and nematode reproduction. At the 10- and 25-mg/plant dosages applied 7 days after the plants were placed in infested soil, the galls remained small and no egg masses were produced. Histological studies of this material showed conspicuous lack of hyperplastic tissue, as well as small, poorly developed giant cells and degenerate female nematodes. The 10-mg/plant dosage applied 14 days after inoculation retarded gall development but did not prevent reproduction.

The effect of root-knot nematode resistance on the incidence of black shank in tobacco. POWELL, N. T., AND C. J. NUSBAUM. Resistance to *Meloidogyne incognita* and *M. incognita* var. *acrita* was incorporated into black-shank-resistant flue-cured tobacco by a backcross procedure. U.S.D.A. breeding line Bel 4-30 was used as the source of root-knot resistance, and Coker 139 and Dixie Bright 101 varieties were the recurrent parents. The second backcross F_1 progeny of each recurrent parent family were separated into root-knot resistant and susceptible segregates by inoculating with *M. incognita*. Vegetative cuttings were taken from each plant, and subsequently divided into 2 groups, 1 of which was inoculated with *M. incognita*. Four weeks after inoculation, plants of both groups were inoculated with *Phytophthora parasitica* var. *nicotianae*. Plants of both recurrent parents were inoculated similarly. In Coker 139 and Dixie Bright 101, the incidence of black shank was much higher among plants inoculated with root-knot nematodes than among those that were not inoculated. Similarly, in the presence of the nematode, root-knot-susceptible segregates were more susceptible to the black shank fungus than were the root-knot-resistant segregates. These studies indicate that the loss from black shank in resistant varieties grown in the presence of both pathogens would be reduced by combining root-knot resistance with black shank resistance.

Attempts to transfer root-knot resistance in tomato by grafting. RIGGS, R. D., AND N. N. WINSTEAD. Root-knot-susceptible (line STEP 174) and resistant (line Hawaii 5229) tomato plants were approach-grafted in pairs and evaluated for their reactions to *Meloidogyne incognita*. Two weeks after the grafts were made the stock of 1 plant and the scion of the other in each pair were removed. Two weeks later, roots of plants of the reciprocal grafts and the resistant and susceptible lines were inoculated, and tops of other plants of the same series were inoculated with a suspension of nematode larvae. Reactions were as follows: 1) susceptible scion on resistant stock showed little or no root galling, but in most cases showed leaf galling; 2) resistant scion on susceptible stock showed severe root galling but no leaf galling; 3) intact susceptible plants showed galling of roots and leaves; and 4) intact resistant plants showed little or no root galling and no leaf galling. These data indicate that the resistance or susceptibility factor(s) was inherent within individual cells in both the roots and tops of plants and either was not translocated or did not cross the graft union.

In-the-furrow application of chemicals as a control for the cotton seedling disease complex. RANNEY, C. D., AND L. S. BIRD. Fungicides and other chemicals mixed with the covering soil at time of planting for controlling the cotton seedling disease complex were tested on sandy and clay soils in Texas in 1957. Some of the materials tested were effective in maintaining higher populations per acre of plants with healthier root systems when compared to the controls. On a state-wide basis 2 materials were particularly effective: a mixture of 1½ lb. 50% captan, 1½ lb. 75% PCNB, and 2 lb. 65% zineb per acre consistently gave a high stand at all locations. Nabam (19%), at 4 qt. per acre, was equally effective on all soils with a pH below 7.3; above this pH, effectiveness was greatly reduced. In preliminary tests at College Station, Texas, the following materials were effective in increasing stands at the .01 level of significance: $Ca(NO_3)_2$ at 10 lb./A; $CaCl_2$ at 10 lb./A; 3 qt. 19% nabam and ½ qt. Ceresan 200/A; 5 lb. 70% maneb/A; 3 lb. 70% maneb and ¼ qt. Ceresan 200/A; 3 lb. 65% thiram and ½ qt. Ceresan 200/A; 2 lb. 50% captan, 2 lb. 75% PCNB, and 10 lb. $Ca(NO_3)_2$/A; and 4 qt. 19% nabam and 10 lb. $Ca(NO_3)_2$/A. In a test applying growth regulators to the covering soil, a 5 ppm solution of the potassium salt of gibberellic acid applied at the rate of 10.5 gal./A was effective in increasing the stand at the .05 level.

A study of the distribution of in-the-furrow applied fungicides. RANNEY, C. D., AND A. M. HILLIS. A study was conducted of the distribution of fungicides applied at planting to the seed furrow as dusts and sprays. A fluorescent indicator was used to determine the placement and degree of dispersion of the fungicide in the furrow and covering soil. The pattern produced by a single nozzle, directed so as to treat the seed furrow and soil moved by the covering device during closing of the seed furrow, was variable. Two or 3 nozzles, arranged so as to treat both the open seed furrow and the soil that tumbled into the furrow, produced a good vertical pattern, but only a narrow ¾–½-inch lateral pattern. When dusts were applied using a Gustafson Chem-Soil mixing tube, a similar narrow vertical pattern was obtained with a conventional rapid-closing device. But when the furrow was gradually closed by the use of several small scratchers, the indicator was distributed in a much wider pattern, approaching that of an ideal distribution. A single dust delivery tube attached so as to blow the dust out through the seed drop tube treated only the bottom of the seed furrow. The study indicates the necessity of using an opening device that produces a seed furrow with a narrow bottom. Results indicate that a surface coverage of a fungicidal material may reduce postemergence losses.

Post-harvest infrared irradiation for the control of gray-mold neck rot disease of onions. ROSBERG, DAVID W., AND H. B. JOHNSON. Infrared irradiation of freshly harvested Excel onions for 6 minutes (6 in. from lamp to onion) reduces post-harvest decay and break-down in storage by the gray-mold neck rot fungus *Botrytis allii* Munn. About 1400 lb. of onions were used in the experiment. Before irradiation, the freshly cut neck of each onion bulb was inoculated with about 25,000 spores of *Botrytis allii* and the onions incubated 24 hours at 85°F and 75% relative humidity. After irradiation, the onions were divided into 2 lots; 1 was stored at ordinary temperatures and the other in refrigerated storage at 34°F for 9 weeks. Total decay in the infrared-irradiated onions stored at 34°F was 4.4% as compared to 98.8% in the non-irradiated control lots. Only 12.3% decay occurred in the infrared-irradiated onions stored at ordinary temperatures, compared with 97.5% in the non-irratiated control lots. Additional evidence indicates that post-harvest infrared irradiation of onions provides a rapid means of artificially curing onion bulbs in seasons when unfavorable weather conditions prevent proper field curing.

Effect of tobacco mosaic virus upon yields of three tomato varieties. SINCLAIR, J. B., AND R. T. BROWN. A field experiment was set up in 5 completely randomized blocks to determine the effect of TMV on yields of 3 tomato varieties. The plots were 20 ft. long, with 10 plants per plot. The varieties used: Grothen's Globe (G.G.), Moreton hybrid (M.H.), and Manalusie (M.). Plants to be infected were inoculated with an isolate of TMV from tomato 8 weeks after field transplanting. Precautions taken to prevent infection of noninoculated plants were successful until the last harvest as shown by weekly assays of harvested fruit. Yields of marketable and cull fruits, as well as total yield, were recorded and adjusted to lb. per acre. Noninoculated plants showed a significantly higher (.01 level) yield when compared with inoculated plants of M.H. and M. in all 3 classes. There was no significant difference in yield between noninoculated and inoculated plants of G.G. in any class. Inoculated M.H. plants gave significantly higher (.01 level) total yields and yield of marketable fruit above the other inoculated varieties. There were no significant differences between any inoculated varieties in the yields of cull fruit. Noninoculated M.H. plants showed a significantly higher (.01 level) total yield and yield of marketable fruit over noninoculated G.G. and M. and of cull fruit over G.G.

Fertilization and ergot of Paspalum. STEWART, ROBERT B. Fertilized florets of Paspalum species are resistant to ergot (*Claviceps paspali* Stevens and Hall), and varieties with high seed set have less ergot. Strains of Dallis grass with yellow anthers normally have high seed set and a correspondingly low ergot incidence; plants with purple anthers have low seed set and high ergot incidence. A cross between these types produced less seed and more ergot than the susceptible parent. This simple relationship is complicated by the fact that the environmental factors that favor seed set may also favor ergot. Drenching Dallis grass inflorescences during anthesis with indoleacetic acid or 2,4-dichlorophenoxyacetic acid significantly reduced the incidence of ergot. It is theorized that in normal fertilization the increased resistance in fertilized florets results from the effect of increased indoleacetic acid upon the physiology of the developing ovary.

Associative interaction among isolates of Botryosphaeria corticis, the cause of blueberry stem canker. TAYLOR, JACK, AND C. N. CLAYTON. Three associative cultural interactions occurred among 15 isolates of *Botryosphaeria corticis* when paired on oat meal agar. Members of one group formed stimulation or raised areas of profuse mycelial growth when paired with members of a second group, but no reaction occurred when an isolate was paired with itself or any member of its group. Isolates of the third group formed an aversion line or a depressed area with scant mycelia when paired with any other isolate. Mycelial transfers from stimulation areas between isolates grew sparsely and irregularly during the first 5–10 days, and in 15 days formed multilocular restricted stromatic mats. Monoconidial colonies from such cultures were all atypical. They all grew, but many did not reach macroscopic size. Some conidia produced 7–8 germ tubes from 1 point. Others had germ tubes that remained short and profusely branched. Growth of subcultures and monoconidial cultures from the original isolates was normal, whereas retarded growth was observed when conidial isolations were made from stimulation zones between paired isolates and from other areas in the same culture. Conidia from the original isolates and from paired cultures contained 8–14 nuclei, averaging 12. Hyphal anastomoses occurred frequently where colonies merged. Sectoring has not been observed.

The effect of flooding on Pythium root rot in nonsterile soil. VAN DER ZWET, T. In previous investigations, severe root rot of sugarcane and corn has been artificially induced

by *Pythium arrhenomanes* Drechs. only when sterilized soil was infested with the fungus. Inability to produce the disease in nonsterile soil made it difficult to evaluate factors influencing the virulence of this pathogen. Since the disease in the field is most severe during wet seasons, when the fields are often flooded, laboratory tests were made of the effect of flooding on disease severity. When soil was flooded for 72 hours, severe root rot was obtained in infested soil not previously sterilized. Corn seedlings grown in nonsterile soil flooded 2 days after planting showed more root rot than those flooded before, at, or more than 2 days after planting. When the time of planting was varied after soil infestation, the corn plants grown in flooded nonsterile soil, planted 2 days after soil infestation, showed 38.5% root infection, compared to 2.5% for the nonflooded check. When continuous and interrupted flooding were compared, the former resulted in a higher percentage of root infection. All of 10 corn varieties tested showed severe root rot symptoms when grown in flooded soil. Percentage of root infection was 18.5–85.0%.

A virus disease complex in soybeans in Arkansas. Walters, H. J. In 1955 and 1956, a virus disease condition was observed in soybeans in the Arkansas River Valley. Losses in most fields were minor, but a total loss occurred in a few fields. A variation of symptoms was observed in the field. Most plants showed a green mottling, with few or no pods and without marked stunting. Other symptoms included proliferation of floral buds, swollen nodes, dropping of immature pods, and failure of plants to mature. By the use of differential hosts and by a study of physical properties, 4 viruses were found occurring naturally in soybean. Soybean mosaic virus, pod mottle virus, and yellow stipple virus were isolated and identified. The fourth virus, still unidentified, was obtained from the complex.

Control of southern blight in tomatoes in northeast Texas. Watkins, G. M., H. C. Mohr, and P. A. Young. At a site in the Red River bottom in Lamar County, northeast Texas, various degrees of control of southern blight (*Sclerotium rolfsii* Sacc.) resulted in 1957 from applications of pentachloronitrobenzene (PCNB), calcium nitrate, or mixtures of PCNB with captan. In a test of dry materials applied to the soil along the row, a comparison was made between PCNB dust at 5, 10 and 20 lb. active per acre, calcium nitrate at 150, 300, 450 and 600 lb. per acre as sidedressing, and granular vapam at 10, 20, 40 and 60 lb. active per acre. Non-treated control plots had a final average loss of 52% of the plants from southern blight, compared with 32% for PCNB at 20 lb., 32% for calcium nitrate at 150 lb., and 41% for vapam at 20 lb. per acre. In a separate test of mixtures of PCNB (as Terraclor 75W) and captan (as captan 50W), applied in water through a standard transplanter as the plants were set, the control plots sustained an average final loss of 71% of the plants, compared with 37.7% in plots in which plants were set with water containing 2 lb. of Terraclor 75W and 2 lb. of captan 50W per 100 gal.

Deleterious changes in tomatoes resulting from irradiation of seeds. Young, P. A. Manalee tomato seeds were treated by the Atomic Energy Commission with 4000–64000 r. of gamma rays or thermal (slow) neutrons for ¼–4 hours. No plants resistant to *Sclerotium rolfsii* were found among the 1015 inoculated R_2 plants, but 3135 plants were examined for visible mutations. Neutrons during 1 hour caused mutations in 4.2% of the R_2 plants; neutrons during 4 hours caused 18.2%; 16,000 and 32,000 r. of gamma rays caused 1.8%; and 64,000 r. caused 0.7% The mutations were deleterious. The natural mutation rate in tomatoes is near 0.002%. The strongest rates of irradiation killed most of the seeds. Neutron treatment caused lethal seedlings with white-purple or yellow cotyledons and stems; dwarfed chlorotic plants with yellow blotches in their leaflets; large green plants with yellowish top leaves but no fruits; leaflets with parallel veins; elongate curled leaflets; entire leaflets on R_2 plants many of whose R_3 descendants were temporarily or permanently topless; and a bud mutation with slender little leaflets with non-necrotic tan spots and fruitless flowers. Gamma rays caused chlorosis, dwarfing, and sterility; many seedlings grew only 1 in. tall, with damaged cotyledons.